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RIBOFLAVIN, LIGHT, AND THE GROWTH OF PLANTS

ARTHUR W. GALSTON

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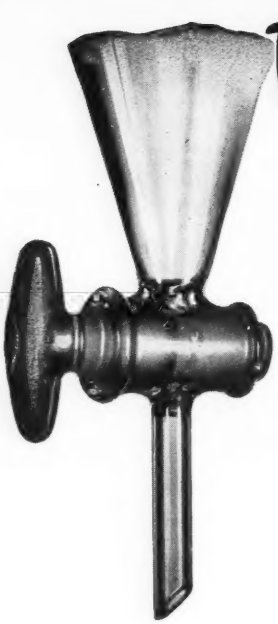

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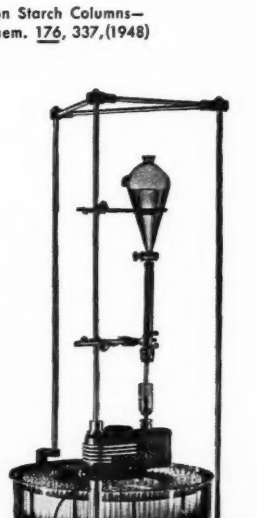

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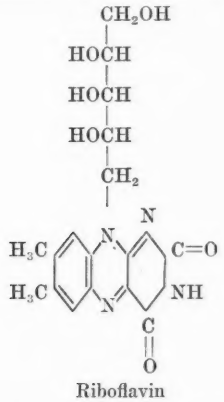
Riboflavin, Light, and the Growth of Plants[1]

Arthur W. Galston

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

RIBOFLAVIN, also known as lactoflavin and vitamin B_2, has been known since 1932, when it was discovered almost simultaneously in the laboratories of Warburg and Szent-Györgyi. Chemically, it is 6,7-dimethyl-9-(1′-D-ribityl)-isoalloxazine). In aqueous solution it is



Riboflavin

yellow, possessing an absorption peak at about 4450 A. It is highly fluorescent, emitting green light when exposed to ultraviolet or blue wavelengths. It is capable of reversible oxidation and reduction, the reduced compound being colorless. It is unstable in light, the type of decomposition it undergoes being dependent on the pH of the medium. In neutral or acid media, the ribityl fraction is split from the molecule, leaving lumichrome, a derivative of alloxazine. In alkaline media, only a portion of the ribityl moiety is removed, leaving an N-methyl isoalloxazine derivative known as lumiflavin.

Biologically, riboflavin is of importance in that it is a constituent of the prosthetic group of various flavoproteins, or "yellow enzymes," which are involved in electron transfers in respiration. Approximately ten yellow enzymes have now been described. These differ greatly among themselves with regard to reactivity, the oxidized form of the various enzymes being reduced by such widely divergent substrates as coenzymes I and II, amino acids, xanthine, and various aldehydes. The reduced form of these enzymes is oxidizable by oxygen, although generally more quickly by other materials, among them methylene blue, fumaric acid, and cytochrome C. The prosthetic group of these enzymes is either riboflavin phosphate or flavin-adenine dinucleotide. In many instances, it has been possible to separate the enzyme into apoenzyme and prosthetic group, with attendant loss of activity. When the two moieties are brought together the enzyme is reconstituted, and the specific activity reappears. It is generally considered that the flavoproteins occupy a position in the respiratory chain of enzymes between the pyridine nucleotide enzymes and the cytochromes.

Almost all of the information concerning riboflavin and the flavoproteins has been obtained by the use of microorganisms and various animal tissues. Although riboflavin is known to be abundant in many higher plants, investigations on its physiological role have been very scanty. In 1937, van Herk (*24*) obtained evidence that flavin enzymes are involved in the prodigious respiration of the *Sauromatum* spadix. The enzyme diaphorase, a flavin enzyme which catalyzes the oxidation of reduced coenzyme I and II by methylene blue, has been found in legume seedlings, potato tubers, and pollen (*16, 18*). Flavoproteins have also been found to stimulate the activity of various dehydrogenases of the *Avena* coleoptile (*1*). These disconnected bits of evidence have not yet been incorporated into a generalized picture of plant respiration.

The fact that riboflavin is a fluorescent pigment has led certain investigators to speculate about its possible role as a light receptor in various organisms. For instance, Euler and Adler (*5*) showed that the retina of the codfish has up to 4175 μg of riboflavin per gram dry weight, a value approximately one hundred times greater than that of tissues like liver and kidney, ordinarily considered flavin-rich. The retinal flavin is mainly dialyzable, and in this free form is highly fluorescent, leading Euler and Adler to attribute to it the role of converting the difficultly

[1] Much of the experimental work herein described has been performed with the aid of Miss Rosamond S. Baker, to whom the author wishes to express great appreciation. The action spectrum herein reported was worked out at the Beltsville, Maryland, laboratory of the U. S. Department of Agriculture, with the cooperation of Drs. S. B. Hendricks, M. W. Parker, and H. A. Borthwick. The carnosine was obtained from Dr. Emil L. Smith and the methylol riboflavin from Dr. Samuel Gordon of Endo Products, Inc.

visible short wavelengths to longer, more easily visible wavelengths. Theorell (*23*) has also speculated on the possible role of riboflavin in color vision. Heiman (*11*) has gathered convincing clinical evidence from various sources to strengthen his belief that riboflavin is involved in vision. He cites papers by Spies and co-workers (*20*), by Pock-Steen (*19*), and by Sydenstricker *et al.* (*21*), in all of which there are reports of effective riboflavin therapy of visual disorders. In addition, photodynamic action of riboflavin *in vivo* has been noted by Heiman and Brandt (*12*) and by Blum (*2*). The photooxidation of ascorbic acid *in vitro* in the presence of riboflavin has been reported by Martini (*17*), Hand, Guthrie and Sharp (*10*), and Hopkins (*13*).

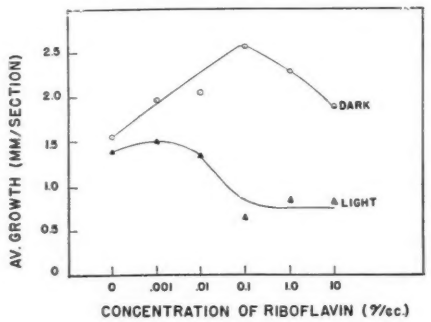


FIG. 1. The effect of riboflavin on the growth of etiolated pea epicotyl sections in darkness and light.

About two years ago, we noticed that the addition of small quantities of riboflavin to media in which plant tissues were being grown results in a slight enhancement of growth if the tissues are kept in the dark, but results in a marked inhibition of growth if they are exposed to light (Fig. 1). Our investigations since that time have led us to the belief that both free riboflavin and flavoproteins may be light-activated, and that such light-activation within the plant may lead to reactions of physiological significance. The evidence for such beliefs is presented below.

Riboflavin-sensitized photooxidations in vitro

The growth in length of plant cells is dependent upon the presence of small quantities of growth hormone such as 3-indoleacetic acid (IAA), generally believed to be metabolically derived from the amino acid tryptophan. The sections of pea stem employed in our original experiments were dependent for their optimal growth upon the presence of 0.1–1.0 μg/ml of IAA in the medium (Fig. 2). We were able to ascertain that the inhibitory effect of riboflavin upon growth of these sections in the light was dependent

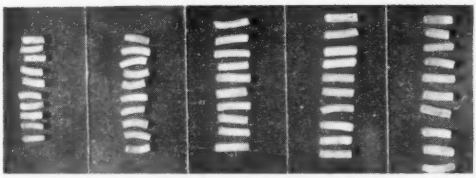


FIG. 2. The effect of indoleacetic acid (IAA) on the growth of etiolated pea epicotyl sections. From left to right, the IAA concentrations were 0 ; 0.01 ; 0.1 ; 1.0 ; and 10 μg/ml.

on the IAA level of the medium. Further investigation of this interrelation (*6*) revealed that riboflavin exerts its effect by causing the photooxidation of IAA in the nutrient medium. Since the growth of the sections is dependent on IAA concentration, such photoinactivation of IAA leads to a marked inhibition of growth of the sections. Thus, we have the paradox of a vitamin's inhibiting growth by destruction of a hormone in the presence of light.

Subsequent investigations of the riboflavin-IAA photoreaction revealed it to be a first order reaction, dependent on the presence of oxygen, and sensitive to pH and temperature. The pH data suggested that the undissociated IAA molecule reacts preferentially. The fact that the riboflavin becomes decolorized under anaerobic conditions made it possible to formulate the following series of stepwise reactions:

$$\text{Rbf} + h\nu \longrightarrow \text{Rbf}^*$$
$$\text{IAA} + \text{Rbf}^* \longrightarrow \text{oxidation product} + \text{Rbf} \cdot \text{H}_2$$
$$\text{Rbf} \cdot \text{H}_2 + 1/2\,\text{O}_2 \longrightarrow \text{Rbf} + \text{H}_2\text{O}$$

This indicates that the light-activated riboflavin (Rbf^*) acts as a hydrogen carrier between IAA and oxygen, and thus is in a role here analogous to the one it plays in respiration. Manometric data indicated that the over-all reaction could be written:

$$\text{IAA} + \text{O}_2 \xrightarrow[\text{light}]{\text{riboflavin}} \text{oxidation product} + \text{CO}_2$$

The nature of the oxidation product is still unknown, but present evidence indicates that it is a condensation product of several cleaved indole rings.

Although such reactions may be studied unambiguously *in vitro*, it is dangerous to extrapolate from the test tube to the living cell. Even if riboflavin, when fed to plant cells, were to produce marked photodynamic effects, these effects might be due to oxidation of substances other than IAA. We therefore undertook a systematic survey of possible biological substrates for such photoreactions.

Using O_2 uptake in an illuminated Warburg apparatus as measurement of the reaction, we found that the amino acid histidine is rapidly photooxidized in the presence of riboflavin, and that the amino acids

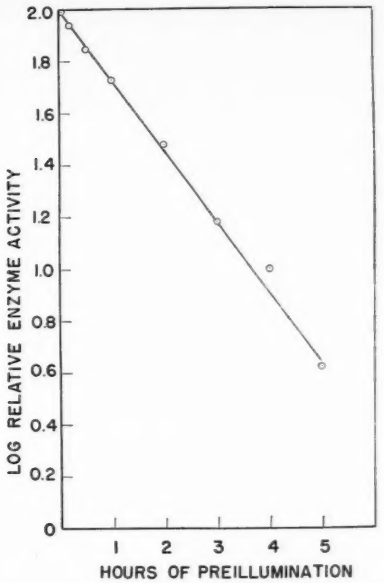


FIG. 3. The time course of photoinactivation of α-amylase sensitized by riboflavin.

tryptophan and methionine, as well as indole-containing compounds, are less rapidly acted upon. Since the amino acids are mainly found in peptide chains *in vivo*, we investigated the influence of their incorporation into peptides on their susceptibility to riboflavin-sensitized photooxidation. In general, we found them to react as well in peptides as in the free state. A good example of this is the dipeptide carnosine, which chemically is β-alanyl histidine. We knew from our experiments on free amino acids that β-alanine does not react in our system, but that histidine does. We found β-alanyl histidine to react, and the manometric data indicated that only the histidine of the dipeptide reacted.

Carrying on investigations into the realm of proteins, we found that all enzymes studied, including α-amylase, tyrosinase, and crystalline urease, are rapidly photoinactivated in the presence of traces of sensitizing riboflavin (*8*). Such photoinactivation also followed simple first order kinetics (Fig. 3). Going to still more complicated protein particles, we investigated, with the aid of Max Delbrück, the photoinactivation of a bacteriophage, T6r. Such virus particles, known to be at least mainly nucleoprotein in nature, were also rapidly inactivated in the presence of riboflavin, but the kinetics of the reaction were indicative of a more complicated "multiple hit" type of phenomenon (Fig. 4).

In view of the current interest in the phenomenon of photoreactivation of ultraviolet-inactivated bacteriophage particles, we tested these riboflavin-photoinactivated bacteriophages for photoreactivatability by incubating duplicate plates in dark and light. No evidence of photoreactivation could be found in our preliminary experiments. This photoinactivation of a bacteriophage is also of great interest because of the chemical similarity between viruses and genes. It seems not beyond the realm of possibility that

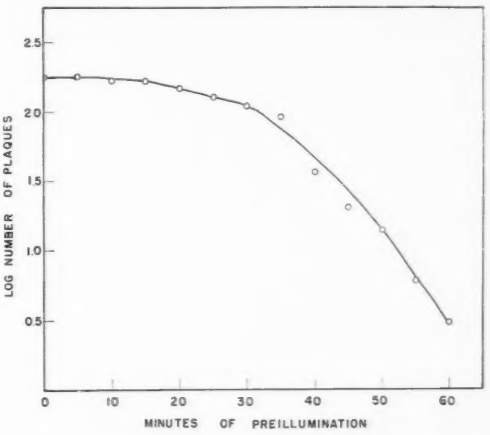


FIG. 4. The time course of photoinactivation of bacteriophage T6r sensitized by riboflavin.

riboflavin may sensitize the genic material of cells to alteration by visible light, and so produce mutations. It should be added, however, that preliminary attempts to produce such a result in the laboratory have been unsuccessful.

Thus it is clear that riboflavin may cause the photochemical alteration of many different kinds of molecules, both large and small. It should also be pointed out that other fluorescent pigments, some of a non-biological nature, are also effective in such reactions. Among such pigments are lumichrome, methylol riboflavins, fluorescein, and eosin.

Light activation of a flavoprotein enzyme

Thus far, we have considered only those photoreactions involving free riboflavin. Since, in most cells, almost all of the riboflavin occurs in flavoproteins, it seemed desirable to investigate the possibility that flavoproteins could similarly be light-activated.

Our first experiments in this field involved the D-amino acid oxidase of hog kidney, and DL-alanine as a substrate. The experiments were conducted in a Warburg apparatus, measurements of the oxygen

consumed by identical systems being made in darkness and in light. We could find no effect of light on the oxygen uptake, and thus had to conclude that light is without effect upon the activity of the enzyme.

We turned next to an enzyme from a plant source, which was not known to be a flavoprotein, but which had the properties of an indoleacetic acid oxidase. This enzyme, described as a heme protein by Tang and Bonner (*22*), may be conveniently studied in breis of etiolated pea epicotyls. We prepared the crude enzyme from this source and, by measuring its activity in darkness and in light, were able to demonstrate a distinct light stimulation of IAA destruction by the enzyme (*9*). The action spectrum for the light stimulation (Fig. 5) is clearly that of a flavin, showing a maximum at 4400 A, a minimum at 4100 A, and a subsequent rise toward the region of the ultraviolet.

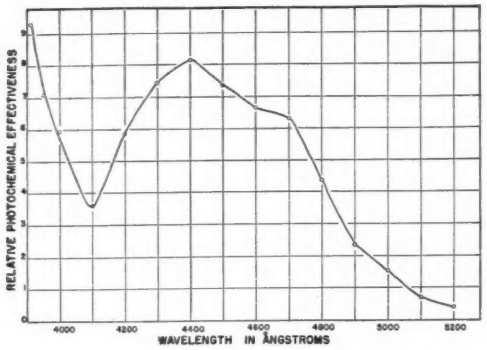


FIG. 5. Action spectrum for the photoinactivation of IAA by etiolated pea brei.

We thought at first that this action spectrum might be due to a light destruction of IAA mediated by free riboflavin, not by a flavoprotein. However, we were subsequently able to demonstrate that the photoreceptor giving the flavin spectrum is largely heat labile, and will not dialyze through a cellophane membrane. Since free riboflavin, riboflavin phosphate, and flavin adenine dinucleotide are all heat stable and dialyzable, the flavin receptor must be a flavoprotein. We therefore concluded that we had demonstrated the light activation of a flavoprotein enzyme. Such a phenomenon has previously been reported for another flavoprotein, xanthine oxidase, by Bernheim and Dixon (*1a*).

Subsequent investigations of this light activation have revealed some interesting features of the mechanism of the light effect. We noticed, as had Tang and Bonner, that dialysis of the crude enzyme greatly increases its activity in the dark, presumably by removal of some dialyzable inhibitor of the reaction. Such dialysis-purified enzymes show very little light activation, as compared with the crude enzyme (Fig. 6). Yet if a colorless solution of inhibitor is added back to the dialyzed enzyme, the inhibition in the dark is reimposed, and this inhibition is once again reversible by blue light, the degree of reversal being dependent on the amount of light relative to the amount of inhibitor present. This led us to the belief that dialysis and light are accomplishing the same effect, i.e., "removal" of an inhibitor. Thus *light activation of this flavoprotein enzyme occurs through reversal of a naturally occurring inhibition,* similar in principle to the light reversal of the CO inhibition of heme enzymes.

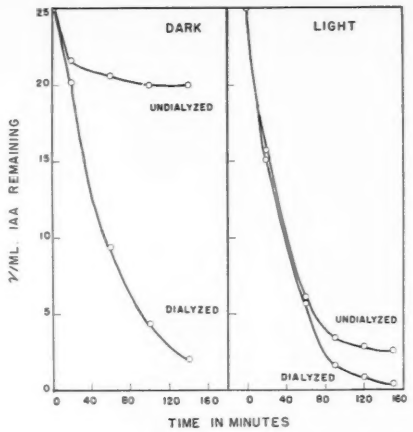
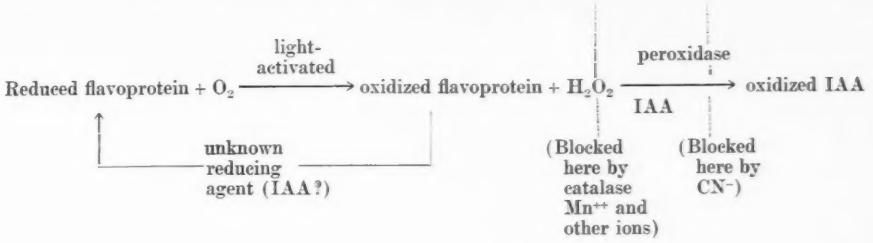


FIG. 6. Relative light activation of undialyzed and dialyzed IAA-oxidase from etiolated peas.

The role of the flavoprotein in this enzyme complex has since been shown to be that of producing H_2O_2, which is then utilized by an IAA-peroxidase (the cyanide-inhibited enzyme of Tang and Bonner). Thus, the reaction destroying IAA is inhibitable by catalase and by various metallic ions which react with H_2O_2. In the absence of inhibitor, H_2O_2 is not limiting to the reaction, and therefore the system is not light-activated. However, in the presence of inhibitor, H_2O_2 is limiting to the reaction and under these conditions, blue light accelerates the reaction, presumably by stimulating the production of H_2O_2 by the flavoprotein system. Our best evidence indicates that the substrate for the flavoprotein is IAA itself; thus IAA oxidation by oxidized flavoprotein appears to lead to the production of H_2O_2, which then is instrumental in the oxidation of more IAA through the peroxidase. The reaction scheme can probably be represented as follows:



The significance of riboflavin-sensitized photoreactions in vivo

Our attention was directed to the possibility that a riboflavin-sensitized photoreaction might be involved in the phototropic curvature of various plant organs. This phenomenon, first studied systematically by Charles Darwin (*4*), is of great historical interest because it led to Went's discovery in 1928 (*25*) of the plant growth hormones. It is now believed that phototropic curvature is the result of a differential growth hormone distribution, leading to a differential rate of growth on the two sides of a unilaterally illuminated plant organ. It can be demonstrated, for instance, that unilateral illumination of the coleoptile (leaf sheath) of a dark-grown grass seedling, such as that of the oat (*Avena sativa*) leads to a lower auxin content on the illuminated side than on the darkened side. In general, it is believed that only the topographic distribution of auxin is affected by light, and not the total amount formed in the tip. This has led to the view that light causes the migration of auxin from the illuminated to the darkened side of the coleoptile, although other explanations involving photoinactivation of auxin and of an auxin-producing enzyme are possible (*7*).

It has been known for many years that blue light is most effective phototropically. In etiolated plants, wavelengths longer than 5200 A are generally entirely ineffective, but in green plants red light may also produce some curvature. As the result of action spectrum determinations for phototropism, such as those of Johnston (*14*), the belief has developed that the receptor pigment for phototropism is a carotenoid, such as β-carotene. In support of this view it has been pointed out that β-carotene is found in abundance in typical photoreceptor organs, both in plants and in animals, and that various carotenoids can sensitize the photoinactivation of auxin-*a* by visible light (*15*). Despite this evidence, we feel that riboflavin or a flavoprotein is a more likely photoreceptor.

In the first place, the absorption spectra for riboflavin and β-carotene are very similar in the visible portion of the spectrum (*9*). Because of the approximate nature of action spectra *in vivo*, it would seem that either pigment could fit the action spectra now available. In fact, most of the action spectra for fungal phototropism (*3*) resemble the flavin spectrum more than the β-carotene spectrum. Second, although β-carotene will sensitize the photoinactivation of auxin *a*, it will apparently not so act toward IAA. The recent evidence of Wildman and Bonner (*26*) seems to indicate that the native auxin of the *Avena* coeoptile is largely, if not entirely IAA. We

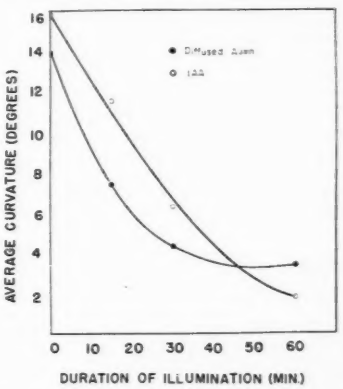


FIG. 7. Relative rates of destruction of pure IAA and of native diffusible *Avena* coleoptile auxin sensitized by riboflavin.

have in addition found (*9*) that riboflavin can sensitize the photoinactivation of the native diffusible auxin of the *Avena* coleoptile, the time course of the disappearance closely resembling that for pure IAA (Fig. 7). Third, riboflavin is abundant throughout the *Avena* coleoptile, including the most light-sensitive apical 0.25 mm, which is relatively free of carotenoids. Fourth, our best evidence indicates that riboflavin, like auxin, is present in the cytoplasm, whereas β-carotene is known to be localized in plastids. It is a little difficult to see how the small quantities of light that suffice to produce phototropic curvatures could be effective if the photoreceptor were

spatially removed from the substrate of the reaction.

Finally, we have the recent experiments of Bandurski and Galston (unpublished) with albino corn. A mutant strain of corn was obtained in which carotenoid pigments were absent, or if present at all, were less than 0.01 the normal concentration. The coleoptiles of such corn, devoid of carotenoids but possessing the normal content of riboflavin, showed approximately normal phototropic curvature when exposed to about 600 meter-candle-seconds of blue light. This experiment does not, of course, disprove the possible participation of carotenoids in normal phototropism, but it does show that other pigments (presumably including flavins) can serve as light receptors for phototropic curvature.

Whether or not a flavin pigment is actually involved in phototropism, it is clear that the reactivity of free riboflavin and of flavoproteins toward certain substrates is markedly affected by light. This fact compels us to examine further the possibility that the ubiquitous flavins may be important photoreceptors in biological systems other than higher plants.

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Technical Papers

Static Electricity Elimination During Sectioning with a Microtome

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Paraffin ribbons of tissue sections become charged with static electricity as they are cut on a microtome, and in dry weather the charge may be great enough to pull the ribbon into contact with the metal parts of the instrument, or other objects, making it difficult or impossible to obtain serial sections without loss. This widespread difficulty has vexed biologists and they have tried in a number of ways to lessen it. For example, they have tried grounding the instrument, and using high frequency generators to ionize the air near the microtome. The first procedure helps, but is inadequate, and the generators are somewhat cumbersome to use and they introduce some hazard of shock.

A simple and efficient solution of this problem is to place a surface on which polonium has been plated about an inch from the edge of the knife. The alpha radiation from the polonium ionizes the air and discharges the static electricity as it forms, leaving an easily handled, limp, uncharged ribbon. A further advantage is that the sections do not tend to stick to the knife facet during cutting, and they are less distorted and compressed when cut without the formation of frictional electricity.

A convenient unit (Fig. 1) consists of a polonium-plated, radiating surface (A) recessed into a rotatable head (B) held on a flexible tube mounted on a base (C). When in use it should be placed with the head about 1 in. from the surface of the specimen block (D) and the emitting surface turned to radiate both the surface of the paraffin block and the ribbon as it forms at the knife edge. This arrangement dissipates the charge formed on the surface of the block on its upstroke and on the ribbon from the friction of cutting. Alpha radiation has little penetrating power to damage the specimen or the sections. The useful life of the emitting surface should be somewhat more than a year from the

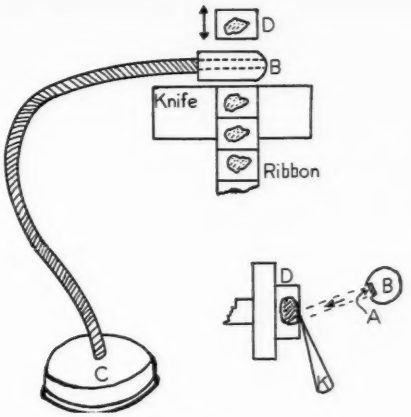

FIG. 1.

time the polonium is plated on the surface. The equipment may be obtained from Mr. Jenkins.

Since alpha rays alone are formed there is no radiation hazard involved from the radiation. Because grease or dirt left on the emitting surface from the fingers will decrease the radiation produced, the polonium strip is protected from contact by being supported within the recess of the head. Should the surface be touched accidentally fingers may become contaminated and should be washed thoroughly with soap and water before eating or smoking, as polonium taken internally is poisonous.

Preparation of Nonprotein Fractions Possessing Adrenocorticotropic Activity from Sheep ACTH Protein

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The possibility that nonprotein material possessing adrenocorticotropic activity may be associated with ACTH protein has been investigated. The stability of the protein hormone to boiling, even in 0.1M HCl, its resistance to destruction by strong solutions of NH_4OH, and the retention of activity even after acid and peptic digestion (*2*), all suggests that the isolated ACTH is indeed an exceptional protein.

Trichloroacetic acid (TCA) precipitation and dialysis have been employed as means of obtaining nonprotein material from an ACTH protein, as prepared by C. H. Li (*2*). All fractions were assayed by the ascorbic acid depletion test of Sayers *et al.* (*4*).

In the usual TCA experiment 20 mg of the ACTH protein was dissolved in 2 ml of water, and 2 ml of ice-cold 10% TCA was added. The solution was stirred and then centrifuged. The supernatant was decanted and the precipitate was suspended in 2 ml of water; 2 ml of ice-cold 10% TCA was again added. The stirred solution was centrifuged, and the supernatant was added to the previous supernatant. The combined supernatants were considered as the TCA supernatant fraction. The precipitate was resuspended and reprecipitated eight to ten additional times, the supernatants being discarded. The final precipitate was washed three times with 4 ml of diethyl ether, and then dissolved in 2 ml of pH 7.5 phosphate buffer. The TCA supernatant fraction was extracted ten times with 4 ml of diethyl ether. The resulting solutions of both the precipitate and supernatant fractions were appropriately diluted, and aliquots were taken for ascorbic acid assay, nitrogen determinations, assay by the repair test of Simpson *et al.* (*5*), and paper chromatography.

FIG. 1 (left). Ascending chromatogram of a 5% TCA supernatant of sheep ACTH protein run in phenol/water, and developed with a 0.2% solution of ninhydrin in ethanol.

FIG. 2 (right). Ascending chromatogram of 4 mg of sheep ACTH protein run in phenol/water, and developed with a 0.2% solution of ninhydrin in ethanol.

It was found that both the supernatant and the precipitate possessed activity by either assay procedure. By the ascorbic acid depletion method quantitative values were obtained which, when compared to the nitrogen content of each phase, demonstrated that the precipitate contained a smaller amount of activity per unit of nitrogen than the original protein. The supernatant, however, showed a markedly increased amount of activity per unit of nitrogen. In various experiments, utilizing different batches of sheep ACTH protein, this has ranged from six to over ten times that of the original protein. In the various TCA supernatants, from 25% to over 40% of the original activity could be recovered, whereas only 4% of the nitrogen was present in the supernatant fractions. Paper chromatograms of both the TCA precipitate and supernatant fractions (Fig. 1), when run

[1] The authors wish to acknowledge the advice of Dr. John H. Northrop, who was kind enough to read and criticize this paper.

in phenol/water, showed fluorescent areas comparable to those obtained with partial hydrolyzates of ACTH protein (*3*), possessing adrenocorticotropic activity.

Intact ACTH protein at a level of 2 mg to 4 mg was then run on paper in phenol/water, as a control, and several spots giving a strong ninhydrin color, together with two distinct fluorescent areas, were observed (Fig. 2). Both fluorescent areas, when eluted, showed adrenocorticotropic activity, while the rest of the paper showed minimal amounts. The fluorescent area of R_f value 0.95 was cut out from the paper and its nitrogen content determined. It was found to contain about 15% to 20% of the nitrogen that was originally placed on the paper.[1]

Tyslowitz (*6*) has reported that phosphotungstic acid will precipitate the activity found in ultrafiltrates of hog pituitary extracts. We have found that phosphotungstic acid supernatants of ACTH protein showed no activity and no fluorescent areas when run on paper in phenol/water.

Dialysis of ACTH protein was performed in distilled water, and against buffers of pH 3 and pH 9, and in 4M urea solution. Twenty mg of the protein was dialyzed in Visking sausage casings in the cold (5° C), for three days against 10 volumes of bathing fluid. The dialyzates in all such experiments showed marked activity. When distilled water was the bathing fluid, for example, a minimum of from 7% to 16% of the original activity could be demonstrated to have passed through the membrane. In such experiments 15% to 20% of the original nitrogen is dialyzable.

We believe that in all the experiments we have performed and reported here the conditions were not such as to lead to the hydrolysis of peptidic linkages. Whether an ACT polypeptide exists in the free state in both the pituitary gland and in sheep ACTH protein hormone, perhaps being adsorbed on the major protein constituent of the hormone, whether there exist both a free peptide and a protein, both of which possess adrenocorticotropic activity, or whether some linkages other than peptidic allow association-dissociation of an active polypeptide and the protein, is not known at the present time. However, if such association-dissociation does exist, it is difficult to understand how single electrophoretic peaks could be obtained over the range of pH from 2.2 to 7.0 (*2*), when it has been shown here that dialyses at pH 3, and against distilled water, along with the 4M urea dialysis, all led to activity in the dialyzate. On the other hand, the inadequacies of electrophoretic analyses of proteins containing small amounts of polypeptide material must be borne in mind. These problems are being investigated at the present time.

Similar considerations to these have been applied to the controversy concerning the protein nature of the posterior pituitary hormones (*1*).

[1] Further experiments performed on additional ACTH protein preparations since this paper was first submitted for publication have shown that not all such preparations give a pattern similar to that shown in Fig. 2. In such cases the TCA supernatant does not show increased activity per unit nitrogen.

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Use of a Wire Recorder for Recording Geiger-Müller Pulses[1]

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In certain medical research studies involving the use of radioisotopes as tracers, it is necessary to perform the studies at two or more locations simultaneously. To have on hand the necessary counting and recording equipment would be both expensive and difficult, particularly since special recording equipment is needed to get the desired result from these studies. The simplest and most inexpensive method of solving this problem has been through the use of the commercial wire recorder. To use such an instrument it is necessary only to provide a power supply for the Geiger-Müller tube, the output of which can be fed into the low gain input of any commercially available wire recorder (e.g., the Webster Model 78).

After the recording is made it is played back in the conventional manner through a pulse limiter and the usual Geiger-Müller counter amplifier, pulse-shaping circuit, and scaler. The end result is exactly like that obtained by using the Geiger-Müller counter directly. The result can easily be checked and adjusted by the use of an oscilloscope which shows the pulse height and width.

The wire recorder affords many other advantages. The wire can be replayed if a check of the result is desired. The same wire can also be used to record voice information pertinent to the experiment in progress. This makes it possible for only one operator to perform the experiment and record the data. At a later time, one person can easily remove all the information on the recorded wire under more favorable conditions than might exist at the time of the original experiment.

With the proper auxiliary equipment it is also possible to record simultaneously on the same wire the counts from two separate Geiger-Müller tubes. This has been tried several times with very good results. A timed switching circuit is employed which switches the output of one of the Geiger-Müller counters on and off at regular timed intervals of 30 sec–1 min. The end result is a record on the wire of the counts from first one tube and then both tubes together. Over the period of time needed for the experi-

[1] This work was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

ment this will average out so that the result from each tube can be found by simple subtraction.

This system has the advantage of simplifying the operation as compared with use of the conventional counting systems. In addition, only one expensive counter amplifier, scaler, and chart recorder is needed to carry on work at several locations simultaneously. In the studies performed in our laboratories, it has been necessary to obtain a record of the counting rate for each minute throughout a 15–20-min period (*1, 2*). This requires a rather elaborate computer and chart recorder. A considerable financial saving and an increase in operating efficiency have been effected by the use of several wire recorders and only the one counter amplifier, computer, and chart recorder.

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Ultraviolet Microscopy and Microspectroscopy of Resting and Dividing Cells: Studies with a Reflecting Microscope[1]

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Recent advances in microscopy, including the development of achromatic and apochromatic reflecting optics (*7, 9, 21, 22*), make it possible to study many fundamental biological problems in a manner not heretofore possible (*2, 3*). In this investigation are described the methods and results of a study of living cells with a reflecting ultraviolet microscope. The limits of monochromatic exposure are estimated in terms of the total ultraviolet radiant energy which does not produce abnormal modification of cells in tissue culture. Ultraviolet microspectroscopy of living cells is carried out within those limits, but subject to the consideration that the extinction values may be affected by one or more of the following factors: (1) the absolute amount of absorbing materials (*12*); (2) the volume in which absorbing materials are distributed (*30*); (3) the loss of light by dispersion or refraction (*12, 30*); (4) the spatial orientation of absorbing materials (*13, 17*); (5) photochemical reactions which may accompany the absorption of light quanta (*25*) during image formation; and (6) chemical changes associated with the oxidation or reduction of absorbing materials themselves (*16*).

Methods

Optical System. The optical system of the reflecting microscope[4] consists of an objective and a condenser, which are two identical lenses of a reflecting-refracting type (*21*). The numerical aperture is 0.72; the focal length is 2.8 mm; and the magnification is 53×. The objective is used alone or with a 4× amplifying-type eyepiece.

Light Source. The ultraviolet light source is a mercury arc (Type A-H4) with the outer bulb removed. In the illuminating system[5] the central image of the arc is focused and projected by aluminized mirrors so that the beam uniformly illuminates just the full aperture of the condenser. When both condenser and objective are in focus, the full aperture of the objective is filled with light in accordance with Köhler illumination.

Wavelength. The selection of wavelength is made by a set of monochromatizing transmission filters for the ultraviolet region (*1, 4, 23*). The principal pairs of mercury lines isolated by three filter combinations and observed with a quartz spectrograph are those at 253.7 and 265.2 mμ, at 275.3 and 280.4 mμ, and at 312.6 and 334.1 mμ.

Photographic Method. The ultraviolet images are recorded by the photographic method, which is the most satisfactory method of ultraviolet image conversion available at present (*24*). According to Caspersson (*13*), only the photographic method is useful for the measurement of the absorption spectra of very small (less than 1 μ) and irregular bodies, although its accuracy, of the order of 5%–10%, is inferior to that of photoelectric methods.

The emulsions which have been selected to compromise the mutually opposing requirements of high sensitivity and high resolution in the ultraviolet region are the Kodak 103-O UV (spectroscopic plate) and the Kodak 1372 (35-mm film). The relative speed of Kodak 103-O UV at 260 mμ is about four times that of Kodak 1372. The resolution of Kodak 103-O UV, on the other hand, is about one-third that of Kodak 1372.

Processing of the negatives is carried out under controlled conditions of temperature, time, and technique which give maximum contrast and reproducible gamma.

Microdensitometry. Microdensitometry of the processed negatives is carried out with a photoelectric microphotometer that has a mechanically positioned film and plate carrier, interchangeable illuminating apertures, and a device for viewing and centering an area for measurement. The radius of the illuminating aperture is chosen so that it will be less than or equal to one-third of the radius of the image area (*12*).

[1] This work was supported by a grant from the American Cancer Society and by funds from the National Cancer Institute of the U. S. Public Health Service.

[2] Fellow in Cancer Research of the American Cancer Society recommended by the Committee on Growth of the National Research Council.

[3] We wish to acknowledge the helpful suggestions of Dr. L. F. Cavalieri and Mr. Mones Berman. We are likewise indebted to Dr. E. H. Land, Dr. E. R. Blout, and Mr. David Grey of the Polaroid Corporation for the opportunity of obtaining the reflecting lenses and the illuminating system which were used in this study.

[4] Designed by Mr. David Grey of the Polaroid Corporation and constructed by the Bausch and Lomb Optical Company under the supervision of Mr. L. V. Foster (*18*).

[5] Designed by Mr. David Grey and constructed by the Research Department of the Polaroid Corporation.

Computation of Extinction. The graphic method of computation (*6, 29*) is used to calculate the extinction ($E_\lambda = \log \frac{I_0}{I}$) for a central portion of an object. I_0 is the intensity of the monochromatic light (λ) incident upon the object and in practice is determined graphically from the photographic density of the image of the empty field in the neighborhood of the object. I is the intensity of the light transmitted by the object and is estimated graphically from the photographic density of the image of the object.

Intensity of Radiation. The intensity of monochromatic radiation is measured by an electronic photometer and a search unit with a phototube (RCA 935). The sensitivity of the search unit and photometer is determined in terms of ergs per second of monochromatic radiation at 253.7, 265.2, 302.2, and 312.6 mμ by comparison with a calibrated thermopile which is exposed to the same intensity of radiation. In addition, for the measurement of the intensity of radiation in small areas of the image plane, or for the comparison of the incident intensity at a reference position with the transmitted intensity at another position, a photomultiplier tube (RCA 1P28) is used in a search unit with a variable aperture. This direct photometric determination of intensity differs from the indirect photographic method, inasmuch as the photographic method measures intensity integrated with respect to time (*32*).

Tissue Culture. The technique of tissue culture departs somewhat from customary procedures. Fragments of tissue, usually numbering three per culture, are placed on a sterile, dry, ultraviolet-transmitting cover glass[6] to which is then added a drop of liquid medium containing *no* plasma. A metal slide with a central aperture is mounted to this cover glass. An additional cover glass, which is sealed to the other surface of the slide, completes the enclosure of the culture.

Ten ml of nutrient medium is made up of two parts by volume of a balanced salt solution (*20*), one part of a solution of penicillin (containing 250 units), one part of a solution of streptomycin (containing 250 γ), two parts of chicken embryo extract, and four parts of human placental serum. Cultures are kept in the lying-drop position at 37° C for 24 hr and then examined for healthy, singly spread cells which have migrated from the explant.

At the time of the microscopic study, the nutrient medium is replaced after several washings with a balanced solution of electrolytes which contains 0.1% glucose, and the culture is placed in the hanging-drop position. There is now liquid contact between the two cover glasses and space for air at the periphery. Except during photography, ultraviolet light is excluded by filters, and the adjustment of the focus of the condenser and the objectives is made in the green light of the mercury arc. Upon completion of the microscopic studies, which require about 30 min, the culture is renourished with its original nutrient medium and placed in the incubator in the lying-drop position until further observation.

This method of preparation and study is applied to fibroblasts and endothelial cells of the embryo mouse, Akm strain (*10*); to a transplantable sarcoma, MA 387 (*5*), of the adult mouse of the Akm strain; to fibroblasts of the embryo chicken; and to fibroblasts of the rat[7] in the 46th generation of passage.

Criteria for lack of injury during the period of observation, which varies from 4 to 18 hr, are the following:

1. The absence of abnormal morphologic change, such as increased optical opacity or refractivity in the nucleus and the cytoplasm, as observed and photographed in a phase contrast microscope (*34*).

2. No increase in the ultraviolet absorption of nuclear material at 260 mμ such as that characteristically associated with the injury of living cells (*8, 26, 30*).

3. The normal continuation of function, such as protoplasmic irritability, migration, and division, as evidenced by continued observations of particular cells.

Results

Ultraviolet Radiation. As a result of a study of the ultraviolet radiation of 25 tissue cultures derived from mesothelium, together with the extensive data of Mayer (*28*) for chicken fibroblasts, an estimate is made of the total ultraviolet exposure tolerated by living cells without injury. The order of magnitude of the intensities used is 1×10^3 ergs/sec/cm², and the time of exposure varies from a fraction of a second to several seconds.

TABLE 1

Estimate of Ultraviolet Radiant Energy Tolerated by Living Cells

Wavelength (mμ)	Radiant energy per unit area (ergs/cm²) Tolerated by living cells	Incident on emulsion (ε_F*)	Incident on cell (ε_C†)	Approx. no. of photographs possible
260	1×10^4	38×10^{-3}‡	0.53×10^3	18‡
275	1×10^4	44×10^{-3}‡	0.62×10^3	16‡
315	$> 1 \times 10^5$	32×10^{-3}‡	0.45×10^3	> 220‡
260	1×10^4	8.7×10^{-3}§	0.12×10^3	83§
275	1×10^4	6.9×10^{-3}§	0.097×10^3	103§
315	$> 1 \times 10^5$	6.4×10^{-3}§	0.090×10^3	> 1110§

* To produce a background density of unity.

† Calculated for a magnification of 53× by the relation, $\varepsilon_C = \varepsilon_F \times M^2 \times \frac{1}{T}$, where M is the magnification (53), and T is the ultraviolet transmission (0.2) of the microscope objective.

‡ Emulsion—Kodak 1372 (35-mm film).

§ Emulsion—Kodak 103-0 UV (plate).

The tolerated exposure, the product of intensity and time expressed as energy per unit area of cell, is 1×10^4 ergs/cm² at 260 and 275 mμ, and in excess of 1×10^5 ergs/cm² at 315 mμ (Table 1, column 2). Within these limits of exposure the living cell undergoes no abnormal

[6] Corning Glass Works, *Vycor*, No. 791.

[7] The original strain was obtained from Dr. H. P. Thompson of the Rockefeller Institute for Medical Research.

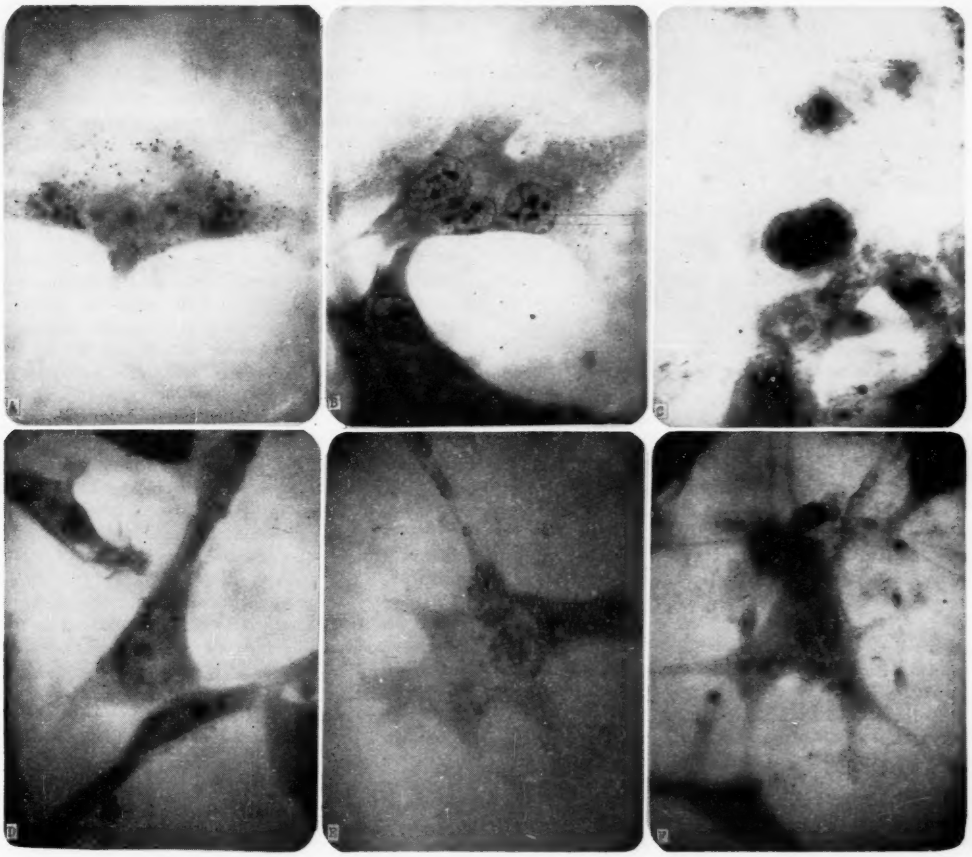

FIG. 1. Ultraviolet photographs of cells: A, rat fibroblast, living; B, rat fibroblast, fixed; C, rat fibroblast, living, mitosis; D, chicken fibroblast, living; E, mouse sarcoma, living; F, mouse sarcoma, living. All photographed at 254–265 mμ. Magnification × 600.

change in size or shape; the transparency of the interphase nucleus is unaltered in visible and in ultraviolet light; the mitochondria are intact; there are no degeneration granules in the cytoplasm; and normal function is not manifestly interrupted. However, in view of technical errors in the measurement of the absolute exposure together with biological variations which are likely to occur in a more extensive study, the limits of exposure are subject to a tenfold variation in order of magnitude.

The foregoing data are useful in predicting the number of photographic images that may be recorded in the ultraviolet region without injury to living cells. In column 3 of Table 1 is given the radiant energy per unit area of emulsion required to produce a photographic density of unity. At 260 mμ the order of magnitude is 38×10^{-3} ergs/cm² for Kodak 1372 and 8.7×10^{-3} ergs/cm² for Kodak 103-O UV. The radiant energy incident on a cell when a photographic image with a background density of unity is formed at a magnification of 53× is given in column 4 of Table 1. At 260 mμ the order of magnitude is 0.53×10^{3} ergs/cm² of cell for Kodak 1372 and 0.12×10^{3} ergs/cm² for Kodak 103-O UV. It is to be noted that the radiant energy incident upon the cell and required for the formation of the photographic image is directly proportional to the square of the magnification at the image plane and inversely proportional to the ultraviolet transmission of the microscope objective.

The approximate number of photographs permitted without exceeding the radiant energy tolerated by living cells is given in column 5, Table 1, and is obtained by dividing the data in column 2 by the corresponding data in column 4. At 260 mμ at a magnification of 53×, approximately 18 photographs may be taken with Kodak 1372 and 83 with Kodak 103-O UV. These results are considered to be first order approximation.

Ultraviolet Photomicrography. Ultraviolet photographs of living cells taken at 260 mμ are shown in

Fig. 1A, C–F, which are positive prints with a magnification of 600×. The low optical contrast (relative to the density of the background) of nuclear structures at 260 mμ is characteristic of the natural condition of resting cells of the type studied in Fig. 1A, C, D, and E.

The typical changes brought about by fixation, for example with acetic acid-alcohol, is indicated by a comparison of the living rat fibroblast, Fig. 1A, with the same cell after fixation, Fig. 1B, and with other cells which had migrated into the field prior to fixation. The ultraviolet absorption of nuclear material at 260 mμ is increased after fixation, and this is due, at least in part, to a decrease in the volume in which the absorbing materials are distributed, and to an increase in the loss of light by dispersion and refraction (*30*). The ultraviolet absorption of cytoplasmic material at 260 mμ undergoes a different change. Formed elements in the cytoplasm such as the mitochondria in Fig. 1A, which dimensionally are at the limit of resolution of 0.2 μ for the objective lens, absorb much ultraviolet light in the living cell. These elements are dispersed in the fixed cell (Fig. 1B), and if initially present in abundance, then contribute to the ultraviolet absorption of the cytoplasmic ground substance.

As indicated in Fig. 1C, there is a characteristically high optical contrast (relative to the density of the background) in nuclear and chromosomal material at 260 mμ in living, dividing cells during certain stages of mitosis. As will be shown, the degeneration of cells is also accompanied by an increase in optical density of nuclear material.

Ultraviolet Microspectroscopy. The extinctions at 260 and 315 mμ in the nuclear and the cytoplasmic material of living and fixed cells are given in Table 2. A range of extinctions is given for nuclear material in order to indicate the order of magnitude of optical heterogeneity. The extinction at 260 mμ for the nuclear material of healthy, resting fibroblasts of the rat, chicken, and mouse, endothelium of the mouse, and sarcoma of the mouse, varies between 0.12 and 0.43. When corrected for the unspecific dispersion of light, as indicated below, the extinction varies between 0.12 and 0.33.

The extinction at 260 mμ for the chromosomal material of rat fibroblasts in mitosis is of the order of 0.97 during stages thus far studied, namely metaphase through anaphase, in which the chromosomes are condensed (*30*) and presumably spiralized in relation to their resting state.

The extinction at 260 mμ for nuclear material in fixed fibroblasts of the rat varies between 0.29 and 0.61, and for nuclear material in degenerating and fixed sarcoma cells, the value lies between 0.59 and 0.82.

The extinction at 315 mμ for centrally disposed nuclear material varies between 0.00 and 0.09. If these values are taken as measures of the unspecific light dispersion, the Rayleigh extinction (*11, 33*) at 260 mμ will be approximately twice the value of the extinction at 315 mμ, i.e., it will vary between 0.00 and 0.18.

The extinction at 260 mμ for the cytoplasmic ground substance of resting fibroblasts and endothelium varies between 0.12 and 0.18, whereas the extinction for cytoplasmic formed elements, such as mitochondria, is undoubtedly much greater but not accurately measurable. After fixation, the mitochondria are dispersed, and the extinction of the cytoplasmic ground substance at 260 mμ is 0.28.

The interpretation of the extinction value, 0.85, at 260 mμ in the juxtachromosomal material of a rat fibroblast in mitosis is deferred until further study. It is the supposition that some of the absorbing material is either derived from mitochondria (*27*) and is dimensionally below the limits of resolution of the microscope, or is related to the formation of the spindle.

TABLE 2

EXTINCTION ($E\lambda$) AT 260 MΜ AND 315 MΜ IN NUCLEAR AND CYTOPLASMIC MATERIAL OF LIVING CELLS AND CELLS IN ALTERED STATES

Culture	Nucleus*		Cytoplasm*		Nuclear diam (μ)	Remarks
	E_{260}	E_{315}	E_{260}	E_{315}		
Rat fibroblasts	0.24–0.36	0.01–0.05	0.18	0.05	16.3 × 14.2	Resting cell (Fig. 1A)
Rat fibroblasts	0.29–0.61	.....	0.28	...	16.1 × 12.1	Same cell, fixed (Fig. 1B)
Rat fibroblasts	0.97†	0.10†	0.85‡	0.10‡	.....	Mitosis (as in Fig. 1C)
Chicken fibroblasts	0.22–0.35	0.00–0.05	0.21	0.00	21.6 × 14.2	Resting cell (Fig. 1D)
Mouse fibroblasts	0.25–0.40	0.07–0.09	0.12	0.00	19.4 × 11.8	Resting cell
Mouse endothelium	0.35–0.38	0.05–0.07	0.17	0.09	19.0 × 14.6	Resting cell
Mouse sarcoma	0.12–0.28	0.00–0.08	0.06	0.05	13.8 × 13.8	Resting cell (Fig. 1E)
Mouse sarcoma	0.37–0.43	0.00–0.05	0.27	0.05	18.0 × 14.2	Resting cell (Fig. 1F)
Mouse sarcoma	0.59–0.70	0.05–0.12	0.40	0.07	18.4 × 11.8	Same cell, degenerated
Mouse sarcoma	0.73–0.82	0.07–0.16	0.62	0.12	15.2 × 10.4	Same cell, dead

* Area of measurement = 1.5 μ².
† Chromosomal material.
‡ Juxtachromosomal material.

The extinction at 260 mμ for the cytoplasmic material of healthy, resting sarcoma cells varies between 0.06 and 0.27 and increases with the degeneration or death of the cell.

As stated previously, the estimate of the total ultraviolet, radiant energy that is tolerated by living cells without manifest injury is based upon a study of a number of tissue cultures derived from embryonic mesothelium, together with the more extensive data of Mayer (*28*) for chicken fibroblasts. The methods of the two

investigations, however, are dissimilar. In the work of Mayer the cells were in a medium containing a plasma coagulum and embryo extract, whereas in the present study at the time of radiation the nutrient medium is replaced by a simple electrolytic solution containing glucose but neither plasma nor embryo extract. Measurements of the ultraviolet transmission of the culture medium made according to an estimate of the amounts and proportions of plasma and embryo extract used by Mayer indicate that this medium transmits only 20% of the incident light at 260 mμ and 275 mμ, and 100% at 315 mμ. Thus, in the original investigation of Mayer, the cells probably received about one-fifth as much radiation at 260 and 275 mμ as had been estimated. Despite these discrepancies in methods, the orders of magnitude for the total radiant energy tolerated by living cells of the type studied at 260, 275, and 315 mμ agree within a factor of ten, which is the probable limit of error of the measurements.

The qualitative aspects of the ultraviolet microscopy of living cells are in general similar to those reported by others (*8, 30*). There are in the literature, however, no quantitative studies of nuclear material such as those described in the present communication.

The type of distributional analysis described by Commoner (*17*) cannot be applied to the present study, for the data are insufficient in number. It is noteworthy, however, that in the nuclear material of healthy, living cells the extinctions at 260 mμ, when corrected for the unspecific dispersion of light, are in the region of 0.3. Furthermore, in mitosis during metaphase and anaphase, the corrected extinctions at 260 mμ in nuclear material increase to values in considerable excess of 0.3. Among several possible interpretations (*17*), such an increase in extinction is suggestive of a change from an oriented to a random distribution of nucleic acids. This change may be related to a decrease in the degree of orientation of the major ultraviolet chromophore, the

$$-C=C-C=N-$$

system of the pyrimidine ring (*15*), in the desoxyribose nucleic acids of chromosomal fibrils which are presumed to be more spiralized in metaphase and anaphase than in the resting state. However, direct observations of the spatial orientation of nucleic acids in the chromosomal material of living cells are not yet reported, and the data for fixed chromosomes (*14, 19, 31*) are inconclusive (*17*).

In summary, an estimate is made of the total ultraviolet exposure tolerated by cells in tissue culture without injury. The value, expressed as energy per unit area of cell, is 1×10^4 ergs/cm² at 260 and 275 mμ, and in excess of 1×10^5 ergs/cm² at 315 mμ. Microspectroscopic studies of living cells are carried out within these limits of exposure. It is noteworthy that in the nuclear material of healthy, resting cells the extinctions at 260 mμ, when corrected for the unspecific dispersion of light, are in the region of 0.3, while in certain stages of mitosis the extinctions in nuclear material increase to values in considerable excess of 0.3.

Addendum

Developments of significance in relation to this work have occurred since this paper was written:

1. Methods have been developed which increase the reflectivity of the mirrors in the microscope objective (Foster, L. V., personal communication).

2. An achromatic monochromator has been utilized to illuminate the microscope with band widths of the order of 50 mμ, or less.

3. Preliminary studies of fixed cells with plane-polarized ultraviolet light gave no evidence of orientation of chromophore at 260 mμ.

4. Spectrographic recordings of the ultraviolet absorption of living cells have been made at *many* wavelengths *simultaneously*, but not without eventual injury to the cell.

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Cataloguing of Infrared Spectra[1]

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A brief method for matching an infrared spectrum, in the 2–16-μ region, of an unknown sample to a large number of known spectra has proved satisfactory for nucleic acid derivatives, and may well be an aid in many of the empirical uses of infrared data. The method is analogous to the ASTM–AXRED X-Ray Powder Pattern Card File as devised by Hanawalt and co-workers (*1*).

For the infrared spectra, the cataloguing procedure is as follows: A smooth base line is drawn on the high transmission side of the absorption bands. This affords a scattering correction. (See Fig. 1 for a solid sample mulled in mineral oil. Visibly transparent samples have a flatter base line.) The extinctions, log ($T_{\text{base line}}/T_{\text{absorption peak}}$), are then determined for the three strongest bands in the region 9.00–15.00 μ. This is primarily the "backbone" vibration region, bands here being more characteristic of the whole molecule than the side group bands of shorter wavelength. The extinction of a band overlapped by other bands is calculated by subtracting from the observed extinction the extinctions of the overlap bands extrapolated. These three bands are then tabulated as to wavelength in order of decreasing extinction, and the ratios of the extinctions of the bands to the extinction of the strongest are tabulated. If two have the same extinction, the one of shorter wavelength is tabulated first. The data for each molecule are put on a 3 in.×5 in. card, the wavelengths of the three strongest bands being prominently placed in the upper left of the card, as with the x-ray cards. Other strong bands (excepting those of mineral oil for mulled samples), chemistry, method of preparation, etc., are recorded on the card (see Fig. 2); the spectrum may be printed on the back. The cards are then filed according to wavelength of the strongest band.

λ(μ)	9.15	9.48	11.97b
E/E₁	1.0	0.7	0.5

1-D arabinosyl uracil
(from Dr. Irving Goodman)
Capillary mineral oil mull

Curve 81198

λ(μ)	E/E₁	λ(μ)	E/E₁
2.83	0.4	9.15	1.0
3.01	0.5	9.48	0.7
5.84	0.3	9.81	0.1
5.98	0.6	10.57	0.3
6.99	0.3	11.04	0.2
7.67	0.3	11.97b	0.5
7.87	0.5	12.28	0.1
8.11	0.5	13.24	0.1
8.30	0.3	15.2bb	0.1
8.65	0.4		
8.98	0.7		

FIG. 2. The infrared file card for 1-D arabinosyl uracil.

To identify an unknown, its spectrum is run and its card tabulated, then the file is searched at the wavelength of the strongest band (in the 9–15-μ region), plus or minus 0.04 μ to allow for wavelength errors. The inclusion of a secondary set of cards, on which the second strongest band is listed first, and filed accordingly, also allows for intensity errors from uncertainties of base line or from instruments of markedly different slit widths. When the three strongest bands are matched, other tabulated bands of the known and unknown are compared and then the spectra are directly matched and possibly rerun under identical conditions. Bands of widths greater than 0.1 μ within 2% of the absorption maxima are designated with a "b," greater than 0.2 μ with a "bb." Improvement in purity or sample preparation may permit resolution of several bands here, so that these values should be considered with caution. Simple mixtures may be identified by successive elimination of bands, though adjacent bands of the components may be unresolved. Chemical compounds, however, have bands in the 9–15-μ region quite different from those of the free components.

As compared to the various punch card systems, such

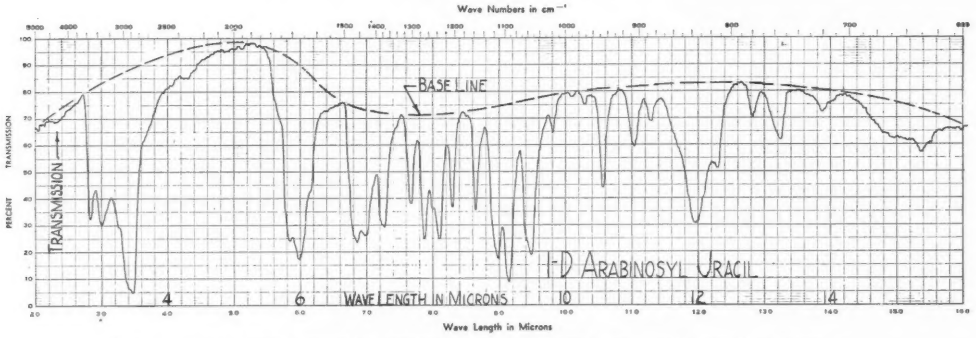

FIG. 1. The infrared absorption spectrum of 1-D arabinosyl uracil, with base line drawn in.

[1] Supported by a fellowship from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

as that described by Wright (*2*), this card file has the advantages of lower cost and greater simplicity of matching spectra. It is not readily searched for subject, author, functional groups, and other detailed information possible to a punch card system, but it is recommended for chemical studies paralleled by infrared identification on a limited class of chemicals, most of which may be identified simply by the three strongest bands in the 9–15-μ region. Such a card file has proved valuable in the rapid identification of over 75 purines, pyrimidines, nucleosides, nucleotides, and nucleic acids, using the mineral oil mull method of sample preparation. Infrared identification of nucleic acid hydrolyzates by this method is now being attempted.

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Colloidal Dispersion of Chloroplast Material[1]

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Chloroplasts, isolated from leaves and placed in a suitable medium, are able when illuminated to liberate oxygen from water (*1*). This photochemical activity is little diminished when chloroplasts are broken into fragments barely visible under a microscope. In order to observe the properties of chloroplast material having submicroscopic particle size, it is necessary to prepare colloidal dispersions of the material. These dispersions are consistently and easily prepared by use of the apparatus described here. It is anticipated that other small particles, such as blood cells, unicellular organisms, and homogenates of animal tissue, for example, can be disintegrated in the same apparatus.

It was found impractical by blending, grinding with sand in a mortar, or using a colloid mill to disperse an appreciable fraction of chloroplast material. Exposure of a suspension of chloroplasts to ultrasonic energy resulted in the disintegration of part of the material to colloidal dimensions. Dispersion was much more conveniently accomplished by using high pressure to force a suspension of chloroplasts through a small orifice. Fig. 1 illustrates the device which was used.

It is made as follows: A 1-in. hole is bored 4 in. deep in the center of a 3-in. steel bar 5 in. long. A smaller hole is bored the rest of the way through the bar and is threaded to take a Hoke steel needle valve. A steel piston slightly less than 1 in. in diam has attached to it a leather washer which fits snugly in the bore of the cylinder. The top ¼ in. of a No. 5 rubber stopper makes a watertight seal between the piston and the cylinder. The leather washer is necessary to prevent the rubber from becoming wedged between the piston and cylinder wall. The top of the piston is drilled and tapped to take a ¼-in. bolt. This provides means for attaching a crosspiece to the piston, in order to withdraw it from the cylinder.

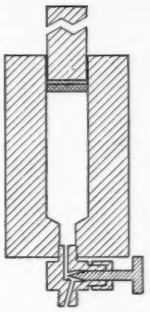

FIG. 1. Dispersion unit.

In operation, 40 ml of a filtered suspension containing 20–30 mg chloroplasts/ml is placed in the dispersion unit. The rubber washer is inserted, broader side down, followed by the piston. The chamber is inverted, the valve opened, and the air forced out. With the valve closed again, the chamber is placed on its base (not shown in the figure) and is put under a 60-ton hydraulic press. After the desired pressure is built up in the chamber, the needle valve is barely opened, so that the liquid runs through at the rate of a few ml/min. During passage of the material through the valve, the pressure is maintained by use of the pump on the hydraulic press. A unit with a smaller bore and with a built-in needle valve for use with laboratory presses has been designed but not tested.

Following passage through the needle valve, the liquid is diluted to contain about 0.5 mg chlorophyll/ml, then it is centrifuged 1 hr at 12,000 × gravity. This empirically determined dilution gives the best yield of material in dispersion at nearly the highest concentration. We consider the chloroplast material which is not sedimented in 1 hr at 12,000 × gravity to be in colloidal dispersion. After separation from the sediment, the dispersion of chloroplast material is dark green, appearing very clear with the light behind it but almost opaque when illuminated obliquely.

Using a pressure of 20,000 psi to force a suspension through the valve, ⅔–¾ of the chloroplast material is colloidally dispersed. In order to do this in one passage through the valve, it was found advisable to freeze the leaves just before isolation of the chloroplasts. Otherwise two passages through the valve were required to attain the same degree of dispersion.

The photochemical activity (*2*) per unit of chlorophyll is about a fourth as great in such dispersions as the activity of intact chloroplasts. Several tests were made to see whether the large loss of activity might be due to the effect of high pressure alone, or to passage of the material through the valve at high pressure. Chloroplast suspensions held 30 min in the chamber at pressures of 5,000, 10,000, 15,000, and 20,000 psi, without passing

[1] The progress of this work was greatly facilitated by a grant from the Research Corporation.

through the valve, lost respectively 7%, 20%, 37%, and 78% of their initial activity. Under these conditions no fragmentation of the chloroplasts was observed. The loss of activity was found to be roughly proportional to the time under pressure. Keeping a chloroplast suspension under a pressure of 20,000 psi for 1 min, 15 min, and 30 min caused the loss of 8%, 44%, and 78% of the original activity. The loss of activity, due to pressure alone, should be small if the time occupied in the pressing operation is made short.

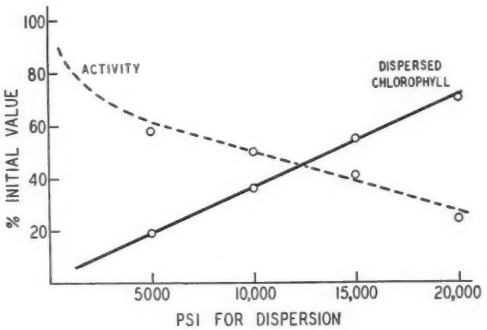

FIG. 2. Completeness of dispersion of chloroplasts and loss of activity at different pressures.

At a pressure of 20,000 psi in each case, chloroplast suspension was forced through the needle valve, set to permit flow at 2, 4, 8, and 16 ml/min. Surprisingly, almost identical yields of dispersed material and losses in activity were observed within this range, that is, the dispersions contained about three-fourths of the total material, with an activity about one-fourth the initial value. The low activity of dispersed chloroplast material seems to be attributable more to its small particle size than to an effect of the pressure used in preparing the dispersion.

Fig. 2 shows the results of forcing chloroplasts through the needle valve at different pressures. In each case the valve was adjusted for a rate of flow between 8 and 10 ml/min. The solid curve shows the percentage of the chloroplast material put into colloidal dispersion, determined by chlorophyll analysis. The broken curve shows the photochemical activity per unit of chlorophyll for the dispersions, compared to the activity of unbroken chloroplasts as 100%.

A dilute suspension of yeast showed about 20% broken cells after passing once through the valve. In collaboration with C. E. Clifton and W. E. Clapper, an experiment was performed in which a dense suspension of *E. coli* was passed through the valve. It showed a several fold increase in glutamic acid decarboxylase activity, presumably due to release of the enzyme from broken cells. The general applicability of the described procedure to a wide diversity of material has not been tested, but we wish here to call to the attention of workers in various fields a simple and possibly useful method for preparing colloidal dispersions of other biological materials.

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Lesions of the Coronary Arteries and Great Vessels of the Dog Following Injection of Adrenalin. Their Prevention by Dibenamine[1]

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The vascular-damaging properties of adrenalin and other vasopressor amines have been extensively investigated. Hueper's review (*3*) indicates that medial necroses and calcifications of the rabbit's and rat's aorta have been the most frequently observed effects. Duff and his associates (*2*) noted also necrosis and hyalinization of the coronary arterioles of rabbits following repeated injections of tyramine. In dogs, consistent arterial changes have not been described following the administration of adrenalin.

The present preliminary note reports the occurrence of segmental necrosis of the coronary arteries, and necrosis and hemorrhage of the media of the pulmonary artery and aorta of the dog following massive intravenous injections of adrenalin. It further reports preliminary experiments indicating that the development of these lesions is prevented by pretreatment with the adrenolytic substance, Dibenamine (*N*, *N*-dibenzyl-β-chloroethylamine).

Four groups of dogs were studied. The first three dogs were given adrenalin intravenously under pentothal anesthesia in sufficient quantity to keep their mean femoral arterial pressures between 220 mm and 280 mm Hg for 30 min. From 8 to 9 ml of standard 1: 1000 adrenalin solution (Parke Davis) was required. These dogs recovered from the anesthesia but died within 24 hr.

In the second series, nine unanesthetized dogs of 10–12 kg were given, on each of three successive days, 1 ml of adrenalin intravenously every 15 min over a period of 1 hr. Their condition remained good throughout, and they were sacrificed between the 4th and 8th day.

The third group of seven dogs were given adrenalin as in the first and second series, but also received Dibenamine hydrochloride,[3] 20 mg/kg intravenously at least 30 min before the adrenalin injection (*4*). Blood pressure determinations carried out on several of these animals

[1] Aided by a grant from the Office of Naval Research, United States Navy.

[2] With the technical assistance of E. Iannucci, P. Integlia, and F. Ferraiolo.

[3] Received through the courtesy of Smith, Kline, and French Laboratories.

made it clear that no rise in systemic arterial pressure occurred during or following the adrenalin injections. At the same time it was noted that the accelerating action of adrenalin upon the heart was not inhibited by the Dibenamine premedication. As a control, two dogs were given Dibenamine hydrochloride alone, 20 mg/kg, intravenously, each day for 3 days.

Autopsies were performed promptly with the termination of each experiment and histologic study of the tissues was carried out.

Animals of the first two groups—which received massive doses of adrenalin alone—showed striking changes in the coronary and pulmonary arteries, aorta, and myocardium. There were no comparable cardiovascular lesions, except for a few scattered areas of myocardial necrosis in the animals of the third group, which received Dibenamine followed by adrenalin. No cardiac or arterial changes were found in the dogs of the fourth group, given Dibenamine alone.

Segmental necrosis of many of the small coronary arteries and arterioles, as well as extensive hemorrhages and necrosis of the pulmonary artery and aorta, was present in the dogs given adrenalin. Hemorrhage was prominent also in the coronary vessels, often occurring, as in the aorta, at the origin of branches. Medial necrosis of the coronary arteries was frequently accompanied by periarteritic cellular inflammatory exudate or by perivascular fibrosis, depending on the age of the lesion. Necrosis of the aortic adventitial vasa vasorum was prominent. An occasional necrotic arteriole of the gastric submucosa was also encountered. There were no renal or cerebral arterial lesions. Interstitial myocardial edema and focal myocardial necroses were observed. Ten of the 12 dogs receiving adrenalin alone had many lesions of the types described. Two had only minimal changes.

The necrosis of coronary arteries observed in the present experiments following injections of adrenalin reproduces faithfully the acute arterial changes often encountered throughout the body in rapidly developing hypertension in man. Similar arterial necroses are well known in dogs and other animals following certain types of experimentally produced renal insufficiency (*5*).

In the present experiment pretreatment with Dibenamine abolished the rise in systemic pressure of subsequent adrenalin injections and prevented the development of arterial lesions. This suggests that in the untreated groups the severe hypertension was a critical factor in the etiology of these changes. Recent experiments of Byrom and Dodson (*1*) have suggested a direct relationship between increased intra-arterial pressure and necrosis of renal arterioles. Experiments are now in progress to see if adrenolytic drugs will prevent the development of acute arterial damage that follows other experimental procedures.

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The Presence in Animal Organs and Human Blood of a Peptide Detected by Paper Chromatography

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Recently, Borsook *et al.* (*1*) reported the existence of a peptide fraction in various tissues of several animal species. This fraction was obtained from extracts of tissues by means of chromatography on starch columns. In this laboratory we have detected a similar or perhaps identical fraction in several organs of the rat, in human blood and tumors, and in Witte's peptone. The fraction was detected first, and isolated later, exclusively by means of paper chromatography (details for the isolation technique to be reported later). Analytical data revealed that this peptide fraction contains 14% nitrogen and is made up of at least the following amino acids: tyrosine, methionine, proline, arginine, hydroxyproline, leucine and/or phenylalanine, alanine, serine, glycine, threonine, glutamic acid, aspartic acid, and lysine.

At present there are insufficient data to warrant the assumption that this fraction is a single peptide. The chromatographic behavior of the fraction does not vary with the tissue of origin. In every case the R_f values were found to be 0.95 in phenol and 0.05 in 2,4-lutidine. Furthermore, the ratio of the value of amino nitrogen after hydrolysis to the value before hydrolysis averages 14.5, which is in close agreement with the value of 14.2 reported by Borsook for "peptide A."

It is conceivable that we are dealing with the same peptide detected by Borsook in various organs of several animal species.

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Book Reviews

Science Is a Sacred Cow. Anthony Standen. New York: E. P. Dutton, 1950. 221 pp. $2.75.

A sprightliness of style, a few well-put and well-deserved cracks at the pompous and the fraudulent, and a fundamentally vulgar line of thinking make this book a minor success of the day. Mr. Standen, chemist and jacket-claimed humanist, discusses the five great branches of science, pays homage to mathematics and the Platonic ideals, and ends by urging the reader to watch scientists carefully against their putative lust for political power. He even draws a laugh out of the matter, for he is no sobersides. Scientists worship a sacred cow, he says, and that is the "most laughable thing in the world." I tried pretty hard to make out that fat, pampered animal, but Mr. Standen's cow seems to me a thin effigy of straw, and he flailed to beat the dust out of her at that.

The technique is simple. He recalls the most obvious prejudices of the chuckling reader. He avoids the great or the difficult like the plague, and gives quotations from a number of more or less obscure elementary texts or books on science teaching. When these are as bad as those things can become, the exposure is on. His arguments appeal to the least reflective, and gloss over everything that might tend to show where the real problems lie. For example, correlations, every reader of *Science* knows, can be and have too often been almost absurdly derived. But Mr. Standen seriously illustrates the meaning of scientific method by pointing out that if one gets tight on whiskey and soda, brandy and soda, and gin and soda, the scientific conclusion is that the common factor, soda water, is the responsible substance. This is really a bit harsh, even on an M.A. in education, who surely uses some controls. And after all it is only the long history of the controls that makes me, with Mr. Standen, believe that ethyl alcohol is the important tenth of the highball. The example cited is by no means unusual. The whole of the book has the same amused and insincere tone.

Physics comes off reasonably well in this ingenious essay. It can't *prove* things (in italics) the way mathematics can, but still it tries. Its results are not just probable, but *very* highly probable. Nearly equal is the same as equal to a physicist. The voice is disapproving, all right, but the words don't say exactly why. Then, in the order named, biology, psychology, and sociology come in for a drubbing, often deserved enough, more often extraordinarily unjust. His strictures on the psychologists who avoid introspective and subjective methods, and on the atomistic sociologists who belabor the evident with the statistician's heaviest clubs are worth reading. But though he speaks many a true word, as he himself gently implies, in jest, the book in sum is a patchwork of facile half-truths and cheap victories.

Mr. Standen says many things about religion which make a pattern. It would have been more candid of him to indicate his own philosophy, and not simply to assert as he does that "the first purpose of science is to learn about God and admire Him through His handiwork." With the many references to Aristotle and his successors, to ghosts and angels, to the saintly and ascetic, Mr. Standen's thinking seems to be recognizable not as that of a humanist but that of a theologian. Since, on page 199, he says that even an indifferent theologian (he so characterizes Plato) is better than a modern scientist, he cannot regard that as harsh criticism.

It is too bad that the good and great men, the fascination and the wonder, the sheer human joys and sorrows that every scientist knows in his science and in its history were ignored. That science can illumine a way of life is lost in this rush of cleverness. There is an acknowledgment to the Long Island Railroad, which gave Mr. Standen plenty of time to write, so he says, by its delays. Either the Long Island has much reformed its schedules since this reviewer last rode to Patchogue, or Mr. Standen is a secret player of canasta, for evidence that the writing of this book took much time is hard to find.

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Brazilian Culture: An Introduction to the Study of Culture in Brazil. Fernando de Azevedo; translated by William Rex Crawford. New York: Macmillan, 1950. 562 pp. $12.50.

This book describes a significant negative contribution of science. Azevedo traces the absence of science through the history of Brazil and shows that it involves scholasticism, colonial economy, slavery, Jesuit education, literary culture, juridical aristocracy, imperial hierarchy, and republican bureaucracy.

The absence of science is not of course complete, and Azevedo gives discriminating consideration to the minor evidences and incidents of science: to the episode of Dutch invasion, to the establishment of technical training under the Empire, and to the interest of scientists of the Northern Hemisphere in the tropical biology and geography of Brazil, as made known by Humboldt, Darwin, Bates, and Agassiz.

The book is not itself scientific except in a sense so broad as to include analytical topical history as a work of science. The author is at his best when dealing directly with Brazilian culture in the spirit of his own cultural heritage and literary style (of which naturally something is lost in the English translation).

There is cause for regret in the fact that the book begins in another style, that of recent social science, partially outmoded. So the author puts his worst foot foremost, with an introduction theorizing on technicalities and five chapters on the "Factors of Culture," in stereotyped order. The first chapter, on "Land and

Race,'' is on that part of the subject in which the author is least at home. The geographic thought is of a previous generation. Environmentalism is retained in practice although disclaimed in theory. Determinism is included by implication. Pages are crowded with place names, meaningless without maps to show their localization and the sweeping lines of movement by which they are related.

An assortment of good pictures and two good specialized maps distributed through the book are given no useful function. They are not referred to in any case and are not presented to show any relation to the text.

If the reader will penetrate beyond Part I and overlook the pictures, he will be rewarded finally with historical understanding of the major absence and minor presence of science in Brazil. For anyone interested in Brazil, the great positive contribution of the book will be found in Azevedo's keen interpretation of the rich nonscientific aspects of the country.

ROBERT S. PLATT

University of Chicago

Experimental Designs. William G. Cochran and Gertrude M. Cox. New York: John Wiley; London: Chapman & Hall, 1950. 454 pp. $5.75.

Application of biometric analysis to research problems has made much progress in the past generation. Experimental design is the culmination of such statistical work; after a little experience in analysis of results, the need for better planning becomes very evident. Plans to insure validity and increase efficiency of experimental work have received increasing attention.

The present textbook has been eagerly awaited for several years, and a preliminary mimeographed version has already proved useful. The book follows the path of useful and usable application of techniques, opened up by Fisher, Yates, Snedecor, the authors, and others. The book is put together substantially and printed clearly. Material heretofore widely scattered, if available at all, is here given organized treatment.

Two short chapters on the philosophy of statistics in experimentation, and a longer one on basic methods of analysis, are followed by ten chapters on specific designs. Completely random designs, randomized blocks, and latin squares (including switch-backs) are first considered. Then factorial designs, confounding, split-plot designs (treated as designs with main effects confounded), quasi-latin squares, and various incomplete block designs are discussed. The last-named group includes various lattices, lattice squares, balanced incomplete blocks, and Youden squares. In each case the discussion includes not only the description and adaptation of the design, but methods for arrangement, randomization, and analysis of results. In numerous cases results from actual experiments, in field and laboratory, are cited in illustration. The extensive tables of plans of treatment combinations, in some cases indexed, should be very useful. The jacket states that 150 plans are listed.

The 14th chapter deals with analyses of series of similar experiments, such as identical tests carried on in several localities; the 15th and last with methods of randomization. References follow each chapter, and a selected bibliography on general principles of design is included.

The more complex designs of limited application receive more space than the simpler and more widely used ones, but this is inevitable because they are more difficult to explain. A little more space might well have been given to the philosophy of experimental proof at the start.

On the whole this volume will be indispensable to forward-looking experimenters and biometricians.

F. M. WADLEY

Department of the Navy

Reviewed in Brief

Surface-Active Quaternary Ammonium Germicides. Carl A. Lawrence. New York: Academic Press, 1950. 245 pp. $6.00.

The author has attempted here to compile the literature of the surface-active quaternary ammonium germicides, incorporating much unpublished work of his own. The contents are grouped into eleven sections. A short introduction is followed by a consideration of the chemical and physical properties of these germicides, and of the problems encountered by the presence of compatible and incompatible materials. Attention is directed to various theories of the mechanism of action, with emphasis on the lack of specific information. There is an excellent discussion of the practical applications of the quaternary ammonium compounds in surgery, in general disinfection, and in industries such as textile plants, laundries, and paper making. A bibliography of 550 references directs the reader to the original sources of material.

It is apparent that a conscientious effort has been made to maintain an impartial attitude in considering the data, although suggestions of a preference for the quaternary substances as antibacterial agents consistently appear. The book is highly recommended as an authoritative survey of our present knowledge in this field.

The Physical Chemistry of Electrolytic Solutions. 2nd ed. Herbert S. Harned and Benton B. Owen. New York: Reinhold Publ., 1950. 645 pp. $10.00.

The six years that have elapsed since the publication of the first edition of this book have not seen any considerable changes in the fundamental concepts of this field. The appearance of revised estimates of certain physical constants has led the authors of this volume to include in an appendix a series of tables representing recalculations based on the new constants. With this exception, the subject matter and organization of this work remain, as before, a standard of excellence.

Association Affairs

AAPG Action on the Visscher Report

When the report of the Special Committee on the Civil Liberties of Scientists was referred to the AAAS Council for action early in 1949, approximately half (130) the Council members voted or submitted written opinions, and of those voting there was a 4-to-1 majority endorsing the report. Among those dissenting were the representatives of the American Association of Petroleum Geologists, who referred the report to their Executive Committee. Following long discussion, involving correspondence with the Washington office of the AAAS and a conference between the Administrative Secretary and W. T. Thom, Jr., one of the representatives of the petroleum geologists on the AAAS Council, the AAPG took the action recorded below.

HOWARD A. MEYERHOFF,
Administrative Secretary

At the annual convention of the American Association of Petroleum Geologists in Chicago, April 24–27, the Executive Committee of AAPG, consisting of five elected officers, unanimously recommended to the Business Committee of AAPG passage of the following resolution in re the report to AAAS of the Visscher Committee on Civil Liberties of Scientists. It was presented together with the explanatory preamble which also follows.

The Business Committee of AAPG consists of 62 members, including representatives from all geographical areas where any considerable number of members of that Association reside. Fifty members were present at the annual meeting of this committee on April 24th. They unanimously approved the resolution and the preamble for presentation to the annual business meeting of the membership of the Association. At the latter meeting, on April 27th, after hearing the preamble, the resolution was adopted, again unanimously.

C. W. TOMLINSON,
Past President

REPORT OF REPRESENTATIVES ON COUNCIL OF AMERICAN ASSOCIATION FOR ADVANCEMENT OF SCIENCE

(From minutes of 35th Annual Meeting of American Association of Petroleum Geologists, April 27, 1950.)

AAAS and Visscher Report

The American Association of Petroleum Geologists some years ago accepted affiliation with the American Association for the Advancement of Science. AAPG has two representatives on the Council of AAAS.

During the presidency of Harlow Shapley, the executive committee of AAAS appointed a "Special Committee on the Civil Liberties of Scientists," of which Maurice B. Visscher was chairman. This committee submitted a report on December 18, 1948, which was summarized in *Science*, issue of August 19, 1949. In that summary it was stated that "The full text was referred to the Council, which voted by an overwhelming majority to publicize the findings."

Our representatives on the AAAS Council, R. J. Riggs and W. T. Thom, Jr., desire the members of the AAPG to know that they voted against acceptance of that report; and that the said "overwhelming majority," because of the large number of councilors not voting, actually constituted only a minority of the council.

The Visscher report specifically recommended that no basic scientific research done for the Government be considered restricted or kept secret; and that no loyalty tests or investigations be instituted against any scientist in Government employ, except those directly engaged in the application of science to the design or manufacture of secret weapons. The report urged substantial restriction of loyalty investigations.

After full discussion with our representatives on the Council of AAAS, the executive committee of the AAPG unanimously recommends, and the business committee of AAPG hereby recommends, that the American Association of Petroleum Geologists pass the following resolution, to dissociate ourselves publicly from the AAAS recommendations:

RESOLUTION

The American Association of Petroleum Geologists recognizes that, as Secretary Acheson has said, the United States is "engaged in a struggle that is crucial from the point of view of the continued existence of our way of life."

The AAPG further recognizes the urgent necessity, under these circumstances, of preventing information of scientific or other nature, vital to our safety, from falling into the hands of a possible enemy.

Totalitarianism has proved itself far more destructive to the liberties of scientists and restrictive of scientific progress than any laws or regulations imposed by the United States in the interest of security or national defense.

The fact that restricted data can be readily and secretly transmitted to unauthorized persons and that the national security can be endangered by even a single disloyal scientist has been amply demonstrated. The Fuchs case offers an example.

The AAPG believes that all loyal citizens of the nation, whatever their category, must be united without reservation in support of the measures deemed necessary by the Federal authorities for the security and defense of the nation. We believe that no loyal citizen, whether scientist or not, should object to investigation of his loyalty. Therefore, although secrecy may for a time impede our scientific progress, we shall abide by such security requirements. We take pride in our readiness, cheerfully and wholeheartedly, to prove our loyalty and patriotism in case of inquiry.

News and Notes

Meeting of the American Physical Society

Vivian A. Johnson

Department of Physics
Purdue University

The American Physical Society held a meeting at Oak Ridge, Tennessee, March 16–18. About 60 percent of the papers dealt with various problems of solid-state physics, there were two sessions on nuclear physics and beta emission, and a few papers were given on miscellaneous topics. The annual business meeting of the Division of Solid-State Physics followed one session. Because of the nature of the program, a large proportion of the approximately 300 physicists who attended the meeting were persons doing research or expecting to do research on problems of solid-state physics.

A single session on Thursday morning, March 16, was composed of nine contributed papers on various metallurgical aspects of solid-state physics. Among the topics described were the use of the semiconducting properties of cuprous oxide to study the oxidation of copper, electron diffraction studies of the orientation of alkali halide crystals grown on various single crystal substrates, relations between magnetostriction measurements and coercive force in permanent magnet alloys, quantitative study of the absorption of hydrogen by zirconium at varying temperatures and pressures, and the use of relaxation phenomena to measure the rate of diffusion of carbon in α-iron.

On Thursday afternoon one session featured invited papers on semiconductors and diffusion. K. Lark-Horovitz, of Purdue University, described the permanent and transient effects produced by irradiating germanium and silicon samples with electrons, deuterons, and neutrons. R. Smoluchowski, of Carnegie Institute of Technology, surveyed the existing models for explaining the mechanism of diffusion in solids and summarized the bearing of recent experimental results upon the acceptance or rejection of these models. C. Zener, of the University of Chicago, advanced a new model of the diffusion mechanism, "ring diffusion," based upon the simultaneous shifts in position of several atoms in the elementary cell; potential energy considerations show such a ring shift to be more probable, in many real crystals, than interchange of two atoms.

A simultaneous session consisted of 14 contributed papers on nuclear physics. The work reported included the use of microwave techniques for the isotopic analysis of nitrogen in ammonia, studies on neutron diffusion in a multiplying medium, neutron energy spectra obtained on bombarding thick targets with deuterons, neutron diffraction by some nuclei with zero spins, the use of nuclear radiations in well logging, the design of a proportional counter to indicate the radiation dosage that would be given to tissue, gyromagnetic ratio measurements for V^{51} and Mn^{55}, measurement of angular correlation between beta and gamma rays in certain decay processes, description of a scintillation counter technique for measuring half-lives of the order of seconds, measured decay schemes for Ba^{131} and Ta^{181}, and the design and test results of the magnet for the Carnegie Institute of Technology synchrocyclotron.

An invited paper session on Friday morning consisted of 35-minute discussions by B. E. Warren, Massachusetts Institute of Technology, on order and disorder in alloys, F. Seitz, University of Illinois, on the statistics of luminescent crystal counters, C. G. Shull, Oak Ridge National Laboratory, on neutron diffraction studies of ferromagnetic and antiferromagnetic substances, and P. W. Anderson, Bell Telephone Laboratories, on the theory of antiferromagnetism.

A parallel session featured contributed papers on a wide range of subjects, including theoretical studies of quantum electrodynamics and the meson mass distribution, piezoelectric effect studies, infrared investigations of diamond, silicon, and germanium, and the production of As_2S_3 glasses transparent in the infrared up to 12 microns.

Another solid-state physics session, on Friday afternoon, consisted of invited papers by H. J. Williams, Bell Telephone, on recent experimental studies of ferromagnetic domains, A. Von Hippel, MIT, on experimental aspects of ferroelectricity, J. C. Slater, MIT, on the theory of ferroelectricity, and N. Bloembergen, Harvard University, on nuclear relaxation in solids.

A second Friday afternoon session was devoted to contributed papers on electron physics and solid-state physics, including reports on electron transit time theory, secondary emission of electrons at oblique angles of incidence, characteristics of scintillation counters, luminescence of color centers in LiF, and production of color centers in quartz.

The Saturday morning symposium on beta emission included a discussion of the theory of forbidden beta ray transitions, by E. J. Konopinski, Indiana University, and a report on the measurement of forbidden beta spectra by P. R. Bell, Oak Ridge, a description of isomeric transitions in nuclei by P. Axel, University of Illinois, and a report on measurement of short-lived isomeric states by F. K. McGowan, Oak Ridge, and S. DeBenedetti, Carnegie Tech, and a talk by E. L. Fireman, Princeton University, on double beta decay.

The other Saturday morning session was devoted to 15 contributed papers on semiconductors, including discussions of the semiconductivity of graphite, electron mobilities in phosphors, the effect of surface states on the resistivity of semiconductors, an a-c circuit for rapid Hall coefficient measurement, purification of germanium by recrystallization, effects of neutron and alpha bom-

bardment of germanium, germanium *p-n* barriers as counters, the growth of germanium single crystals, the technique of cutting Ge filaments, the quantum yield of electron-hole pairs in Ge, relation of noise level to fluctuations in hole concentration in Ge, and magnetoresistance in Ge.

An over-all view of this meeting shows an intense interest in solid-state physics, particularly in investigations of the elementary semiconductors such as germanium and silicon. Entirely new developments reported in this field include the experimental discovery of directional anomalies in the magnetoresistance effect in Ge single crystals and the discovery that Ge barriers can act as counters for radiation detection. The theory of diffusion in solids was seen to be in a state of flux, characterized by the obtaining of data, from recently designed experiments, that tend to favor the "vacancy-jump" model of diffusion over the models based upon motion through interstitial positions or motion by interchange of atoms; in addition, a good case was made for a new model, "ring" diffusion, describing the motion of atoms through a solid as the result of successive rotations of rings of three, four, or more atoms within an elementary cell. Also worthy of mention as a new development is the discovery of a new group of glasses (As_2S_3) transparent in the infrared up to 12 microns.

Roy Hertz, chief of the Endocrinology Section, National Cancer Institute, and assistant clinical professor of medicine at George Washington University Medical School, Washington, D. C., will speak before the Ciba Foundation for the Promotion of International Cooperation in Chemistry and Medicine, in London, July 10. Dr. Hertz will describe experimental and clinical studies on endocrine aspects of cancer now in progress at the cancer institute and the university hospital.

Roger Conant Crafts has been appointed head of the Department of Anatomy at the University of Cincinnati College of Medicine. Dr. Crafts, who has been a member of the Department of Anatomy at Boston University School of Medicine, will succeed the late Joseph L. Schwind, who died May 21, 1948.

Louis M. Hellman has been appointed professor of obstetrics and gynecology at the College of Medicine of the State University Medical Center in New York City, effective August 1. Dr. Hellman is now associate professor of obstetrics at Johns Hopkins University.

Dale A. Porter has been appointed director of the U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama, to succeed **A. H. Groth**, who resigned last September. Dr. Porter has been parasitologist at the laboratory since it was established in 1938, and has been assistant director since 1947.

John E. Dougherty has joined the staff of the Los Alamos Scientific Laboratory's Weapons Division. Dr. Dougherty has been engaged in work on the design, construction, and testing of the new synchrotron of the Engineering Research Institute at the University of Michigan.

Helen Peak, professor of psychology and head of the department at Connecticut College, has been appointed Catherine Neafie Kellogg Professor of Psychology at the University of Michigan. Dr. Peak is the first to fill this chair, for which the endowment fund was begun more than 50 years ago.

Sanborn Partridge, of Yale University, has been appointed instructor in structural geology in the Department of Geology, University of Kansas. Dr. Partridge will also be in charge of the university's new seismological station.

Visitors to U. S.

Sir Richard Southwell, rector of the Imperial College of London, will lecture at the Michelson Laboratory, Naval Ordnance Test Station, Inyokern, California, on June 13. He will speak on relaxation methods.

Hans Kopferman, professor of physics at the University of Göttingen, Germany, and at present visiting professor at Massachusetts Institute of Technology, addressed a physics symposium at Princeton University last month.

Recent visitors at the National Bureau of Standards were **L. R. Hällebo**, development engineer, Air Board, Royal Swedish Air Force, Stockholm; **P. L. Larger** and **M. A. Tenant de la Tour**, engineers with the Chambre Syndicale des Fabricants de Tubes d'Acier, Paris; **W. E. K. Middleton**, National Research Council of Canada; **Y. Nayudamma**, chemist with the Government of Madras, India; **W. Six**, chief engineer of the Philips-Eindhoven Research Laboratories; **J. M. Unk**, of the same firm, and professor at Technische Hogeschool, Delft, Holland; **Bjarne Bassoe**, secretary general, Norwegian Society of Civil Engineers, Oslo; **V. D. Burgmann**, officer in charge of wool textiles, Australian Commonwealth Scientific and Industrial Research Organization, Sydney; **G. Goudswaard**, director of the permanent office of the International Statistical Institute, The Hague; **R. D. Neale**, professor of electrical engineering, Canterbury University College, Christchurch, New Zealand; **A. Parker**, director, Fuel Research Center, Department of Scientific and Industrial Research, London; **O. G. Sutton**, head, Department of Mathematics and Physics, Military College of Science, Swindon, England; and **W. J. Van Dalfsen**, head, Fiber Research Institute, Endschede, Holland.

César Gómez, former director of the National Hygiene Institute in Bogota, Colombia, is in the U. S. visiting medical laboratories.

Grants and Awards

The Biochemistry Branch, Biological Sciences Division, of the Office of Naval Research recently announced the following contracts: University of Indiana, *W. J. van Wagtendonk*, nutrition, serology, and genetics of *Paramecium aurelia*;

Fordham University, *F. F. Nord*, the conversion of carbohydrates to fats by pigments in molds; College of Puget Sound, *R. D. Sprenger*, isochroman chemistry; Cornell University, *H. A. Scheraga*, flow birefringence in macromolecules; University of Detroit, *E. B. Gerheim*, serum proenzyme; University of Cincinnati, *J. H. Howard*, proline and hydroxyproline; Western Reserve University, *A. M. Potts*, methanol metabolism and visual enzymes; Creighton University, *H. C. Struck*, methylene blue and mammalian erythrocytes; University of Utah, *L. T. Samuels*, synthesis of hormones and enzymes in the mammalian body; Loyola University at Chicago, *M. B. Williamson*, amino acids in wound healing; Harvard University, *Paul Doty*, protein interactions; University of California, *E. A. Adelberg*, the synthesis of isoleucine, valine, and threonine.

Melvin DeGroote, vice president of the Tretolite Company, manufacturing chemists of St. Louis, Missouri, received the **Ohio State University's Lamme Medal** on June 9. The award is given annually to a graduate in recognition of achievement in engineering or technical arts.

The 1950 Progress Medal of the Photographic Society of America has been awarded to Loyd A. Jones, head of Kodak Research Laboratories' Physics Department. The award, instituted in 1948, is made annually for outstanding contributions to photographic science and practice.

Colleges and Universities

The University of North Carolina officially opened its new $175,000 Venereal Disease Experimental Laboratory at Chapel Hill on May 16. The laboratory, which was begun two years ago, was built with the cooperation of the State Board of Health and the U. S. Public Health Service. An attempt to produce a vaccine for immunization against venereal disease will be one of the projects of the laboratory.

Carnegie Institute of Technology has established a new professorship in chemistry, made possible by a grant of $15,000 a year by the Gulf Oil Corporation. The new chair will be named for Benjamin Silliman, pioneer in the chemistry of petroleum refining, and will be occupied by Frederick D. Rossini, chief of the Thermochemistry and Hydrocarbons Section, National Bureau of Standards, who will join the faculty as head of the Chemistry Department on July 1.

A research institute for the study of alcoholism, physical rehabilitation, and chronic diseases has been established at the **University of Buffalo** by a tripartite arrangement of the U. S. Public Health Service, the Illinois State Health Department, and the university. The institute will be operated by the university's Medical School, aided by funds provided by the state health department. An amount not to exceed $200,000 has been provided for the current year, beginning April 1, 1950. Buildings in Buffalo have been provided by the U. S. Public Health Service.

The University of Minnesota's Department of Geology has been granted a bequest of approximately $18,000 from the estate of Junior Hayden, Minneapolis naturalist. The fund will be used mainly to promote general education in the earth sciences. During the past two years Mr. Hayden had contributed nearly 2,000 Kodachrome slides of geologic subjects to the department.

Summer Programs

The Institute for Teachers of Mathematics, sponsored by the Association of Teachers of Mathematics in New England, will be held at Tufts College, Medford, Massachusetts, August 22–29. The program will include lectures on the latest developments in pure mathematics and the applications of mathematics. Further information may be obtained from Janet S. Height, Wakefield High School, Wakefield, Massachusetts.

The second annual Oak Ridge summer symposium, devoted this year to quantum and inorganic chemistry, will be held August 21–31, under the joint sponsorship of Oak Ridge National Laboratory and Oak Ridge Institute of Nuclear Studies. The principal speakers will be Peter Debye, Cornell University; Henry Eyring, University of Utah; Herbert S. Harned, Yale University; Linus C. Pauling, California Institute of Technology; and George Scatchard, Massachusetts Institute of Technology. There will be no admission fee. Additional information may be obtained from the University Relations Division, Oak Ridge Institute of Nuclear Studies, P. O. Box 117, Oak Ridge, Tennessee.

Meetings and Elections

The 27th Annual Plant Science Seminar will be held at the Massachusetts College of Pharmacy, Boston, Massachusetts, August 24–30. Members desiring to present papers may write to either Dr. Heber W. Youngken, Sr., Local Secretary, or Dr. Maynard W. Quimby, Chairman, Massachusetts College of Pharmacy, Boston 15.

The American Oil Chemists' Society elected the following officers for 1950–51: president, John R. Mays, Jr., Barrow-Agee Laboratories, Inc., Memphis; vice president, A. E. Bailey, Girdler Corporation, Louisville, Kentucky; secretary, H. L. Roschen, Swift and Company, Chicago; and treasurer, J. J. Vollertsen, retired from Armour and Company, Chicago.

The American Society for Pharmacology and Experimental Therapeutics elected the following officers at its meeting in Atlantic City, April 17–21: president, Carl F. Schmidt, University of Pennsylvania; vice president, McKeen Cattell, Cornell University Medical College; secretary, Harvey B. Haag, Medical College of Virginia; and treasurer, K. K. Chen, Lilly Research Laboratories.

The Population Association of America elected the following officers at its annual meeting at Princeton, April 29 and 30: president, Philip M. Hauser, Department of Sociology, University of Chicago; first vice president, Dorothy Thomas, Wharton School, University of Penn-

sylvania; second vice president, Margaret Hagood, Division of Farm Population and Rural Life, Bureau of Agricultural Economics, Washington, D. C.; treasurer, John Durand, Social Affairs Department, United Nations, Lake Success; and secretary, Henry Shryock, U. S. Bureau of Census, Washington, D. C.

The American Physiological Society, at its annual meeting in Atlantic City, April 17–21, elected H. C. Bazett, of the University of Pennsylvania, president of the society for 1950–51. Others elected to office were: president elect, D. B. Dill, Army Chemical Center, Maryland; councilors, E. F. Adolph, University of Rochester, and F. A. Hitchcock, Ohio State University; secretary-treasurer, R. W. Gerard, of the University of Chicago.

Deaths

Raymond C. Benner, 68, died April 20 while attending the Electrochemical Society convention in Cleveland, Ohio. Dr. Benner had served as consultant engineer for several government agencies, including the Office of Naval Research, following his retirement in 1945 as director of research for the Carborundum Company, Niagara Falls, New York. He held more than 250 patents in the fields of abrasives, refractories, electricity, chemistry, mining, and mechanics.

Howard S. Reed, professor emeritus of plant physiology at the University of California at Berkeley, died May 12 of a heart attack. He was 73 years old. Dr. Reed became a member of the university of California faculty in 1915 and retired in 1946. He was an authority on the history of plant sciences, including pre-Columbian botany.

Helmut L. Bradt, 32, professor of physics at the University of Rochester, died May 24 at Brooklyn, New York, following a lung operation. In collaboration with Bernard Peters and Morton F. Kaplan, of Rochester, Dr. Bradt had recorded photographic evidence in January of a new atomic particle, the neutral meson. He had been appointed associate professor of physics at Stanford University, California, effective this month.

Geochemica Acta, a new international journal devoted to the chemistry of the earth and the cosmos, is soon to be published in London by Butterworth-Springer, Ltd. F. Earl Ingerson, chief of the Geochemistry and Petrology Branch of the Geological Survey, will serve as American editor of the journal. European editors will be F. A. Paneth, Durham, S. R. Nockolds, Cambridge, L. R. Wager, Durham, for England; F. E. Wickman, Stockholm, for the Scandinavian countries; C. W. Correns, Göttingen, for the German-speaking countries. Papers on geochemistry and cosmochemistry will be published in English, French, or German, with English summaries.

The Fan Memorial Institute of Biology, Peiping, China, has been incorporated with the botanical institutes of Academia Sinica and the National Academy of Peiping to form a new Institute of Plant Taxonomy, located in the Botanical Institute of the National Academy of Peiping. Dr. Hsien-hsu Hu, of the institute, has established a separate laboratory at 22 Shi-h Fu Ma Ta Chieh, West City, Peiping, China.

A bibliography on cortisone and ACTH, prepared by the New York Academy of Medicine, is available to physicians on request from Janet Doe, Librarian, New York Academy of Medicine, 2 East 103rd Street, New York City.

Two of the nine sections of *A Glossary of Terms in Nuclear Science and Technology,* being prepared by committees under the general sponsorship of the National Research Council's Glossary Conference, have been published in preliminary edition for criticism by workers in the field. The available sections are on chemical engineering, biophysics, and radiobiology. Other sections in preparation are on general terms, reactor theory, reactor engineering, chemistry, instrumentation, isotope separation, and metallurgy. The *Glossary* will be published in one volume after the trial period. The published sections, priced at 60 cents each, may be obtained from the publisher, The American Society of Mechanical Engineers, 29 West 39th Street, New York City.

An advisory committee in biology, the third of several committees planned to provide advisory services to the Office of Naval Research on research proposals, has been established in the American Institute of Biological Sciences of the National Research Council. Members of the new committee are: chairman, Max Kleiber, professor of animal husbandry, University of California; H. B. Steinbach, professor of zoology, University of Minnesota; A. W. Martin, head of the Department of Zoology, University of Washington; Victor Twitty, head, Department of Biological Sciences, Stanford University; Froelich Rainey, director of the University Museum, University of Pennsylvania; Marston Bates, Rockefeller Foundation; Daniel Merriman, director, Bingham Oceanographic Laboratory, Yale University; and Theodore Jahn, head of the Department of Zoology, University of California at Los Angeles.

Make Plans for—

American Institute of Electrical Engineers, summer and Pacific general meeting, June 12–16, Huntington Hotel, Pasadena, California.

American Malacological Union, June 14–16, Chicago Natural History Museum, Chicago.

Conference on Terramycin, Section of Biology, New York Academy of Sciences, June 16–17, Barbizon-Plaza Hotel, 101 West 58th Street at Sixth Avenue, New York City.

American Astronomical Society, 83rd meeting, June 18–21, Kirkwood Observatory, Bloomington, Indiana.

American Society of Heating and Ventilating Engineers, June 19–21, Lake Muskoka, Ontario.

Chemical Institute of Canada, annual meeting, June 19–22, Royal York Hotel, Toronto.

American Society of Mechanical Engineers, semiannual meeting, June 19–23, Hotel Statler, St. Louis, Missouri.

For study of submicroscopic structure by X-ray Diffraction, Kodak offers 6 films

Choice of the best film for a specific problem depends upon the relative importance of film speed, contrast, and graininess—as well as the quality of the radiation. The table shows the characteristics of six Kodak Films suitable for direct x-ray exposure in diffraction work.

EASTMAN KODAK COMPANY
X-RAY DIVISION
Rochester 4, N. Y.

Kodak Films for X-ray Diffraction	Relative speed, K radiation			Contrast	Order of increasing graininess
	Cr	Cu	Mo		
Kodak Industrial X-ray Film, Type K	150	130	110	high	3
Kodak No-Screen X-ray Film	100	100	100	low	3
Kodak Industrial X-ray Film, Type F	85	60	40	low	2
Kodak Single Coated X-ray Film—Blue Sensitive	80	45	25	low	2
Kodak Photoflure Film—Blue Sensitive*	80	45	25	low	2
Kodak Industrial X-ray Film, Type A	22	17	14	high	1

*Kodak Photoflure Film—Blue Sensitive is available only in 100-foot rolls of 70-mm. width.

For each radiation quality, Kodak No-Screen X-ray Film has arbitrarily been assigned the speed value of 100 (at a density of 0.3 above base density plus fog). It is not valid to compare film speeds for different radiation qualities. Normal development is 5 minutes in Kodak Rapid X-ray Developer at 68° F. (20° C.) with 5 seconds of agitation each minute.

For a notebook-size version of this chart and further information, write for the new free booklet, "Kodak Materials and Accessories for Industrial Radiography."

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Personnel Placement

POSITIONS WANTED

Academic Position in Biology. Eight years experience in University teaching and research. Doctorate in parasitology. Rocky Mountain Region preferred. Box 187, SCIENCE. X

Academic Position with opportunity for research is desired by Ph.D., age 29, with broad training including four years of industrial research experience and one year post-doctoral fellowship. Organic chemistry, pharmaceuticals, natural products. Box 189, SCIENCE. X

Anatomist-Biologist: Summer or new contract, teaching, research, writing or representation. Experienced college, graduate, professional school histology, physiology, botany, zoology. Columbia degrees and teaching. Box 184, SCIENCE. X

Bacteriologist: Ph.D., June. Biochemistry, Dairy Bacteriology, University Fellow, Sigma Xi, Phi Sigma. Desires industrial fermentation or pharmaceutical research. Box 190, SCIENCE. 6/23

Biochemist, Ph.D., 26, veteran. Graduate large Eastern University. Degree minors: organic chemistry and animal physiology. East or Midwest location desired. Available immediately. Box 197, SCIENCE. 6/23

Biophysicist: M.D. Desires position with group developing electronic techniques for medicine and biology. Age 38. 12 years with Navy Medical Corps in internal medicine, aviation medicine, research, teaching and industrial toxicology. Publications. Box 175, SCIENCE. 6/9

Biophysicist—Recent Ph.D., University of California. Background: plant science, biochemistry, carbohydrate chemistry. Competent electron microscopist experienced in replica techniques. Desires teaching and/or research position. Box 185, SCIENCE. X

Chemist. M.A. Male. Electrochemistry, organic, instrumentation, machine-shop experience, desires industrial position. Excellent references. Location immaterial. Box 196, SCIENCE. X

Chemist; Ph.D.; experience principally in biochemical and organic research in industry; experienced in teaching; ten years, director, bioassay laboratory; has done considerable research on the preparation of vitamin D and hormones; for further information, please write Burneice Larson, Medical Bureau, Palmolive Building, Chicago. X

Geneticist. Woman. Recent Ph.D. Interested in teaching or research, particularly with microorganisms. Box 177, SCIENCE. 6/9

Geology Instructor, M.S., 15 years teaching experience, last 6 in college, desires similar position; East; can organize geology department. Box 186, SCIENCE. X

M.A,. Physical Chemistry, Columbia, 1950. Some biochemistry. Three years research with radioisotopes. Laboratory instructor food chemistry. Box 191, SCIENCE. X

Medical Editor: Ph.D., age 40, member of medical school faculty and editor of medical journal. Desires position as editor or editorial associate in field of biological and medical sciences. Box 198, SCIENCE. X

Physician-Anesthesiologist, well trained, N. Y. license. Vet., desires research position; do some clinical work, consider fellowship for training period. Box 192, SCIENCE. X

Physician—Ph.D. (Hygiene and Bacteriology). Under 40. Wide experience teaching, research and administration. Bacteriology, immunology, and public health, especially tuberculosis control. Publications. Box 193, SCIENCE. X

Physicist: M.A., considerable teaching experience. Available due to junior college closing. Box 194, SCIENCE. X

Zoologist: B.S. Wildlife Management & General Biology, Texas A & M; M.S. Entomology & Invertebrate Zoology in August; 1 semester lab instructor Botany; some school journalism. Age 27. Interest: teaching or research; prefer Southwest. Write R. S. Robertson, Box 2355, College Station, Texas. X

Zoologist: Woman. M.S. in Parasitology and Medical Bacteriology; 58 hours toward Ph.D.; two years teaching and two years hospital laboratory experiences. Desires laboratory or teaching position in New York City or vicinity. Box 188, SCIENCE. X

Personnel Placement

POSITIONS OPEN

Large Community blood center now moving into new building with excellent research facilities desires young person (M.D. or Ph.D.) interested in research as a career to direct its research program. University teaching appointment can be guaranteed to successful applicant. T. J. Greenwalt, M.D., 925 West Wells Street, Milwaukee 3, Wisconsin. X

Parasitologist: B.S. or M.S., assistant in Experimental Research Laboratory, northern New Jersey pharmaceutical organization. Box 195, SCIENCE. X

Positions Open:
(a) **Microbiologist;** Ph.D.; fundamental training in fermentations, soils and allied biochemistry required; research laboratories, large industrial company; Pacific Coast. (b) **Assistant professor of anatomy;** to direct course in embryology, assist in histology and neural anatomy; will consider Ph.D. in zoological fields if experienced in teaching histology and embryology. (c) **Research assistant;** university department of pathology; preferably physician or Ph.D.; sound background biology or chemistry; principally cancer research; around $6000. (d) **Biochemist** qualified to direct division, large general hospital; Gulf Coast; $6000. (e) **Endocrine research chemist;** capable of doing endocrine assays; 18-man clinic operating own hospital of fairly large size; university affiliations; East. (f) **Bacteriologist** to supervise bacteriology, serology, mycology, parasitology, blood bank; duties include teaching, participating in resident training program; large teaching hospital. S6-2 Burneice Larson, Medical Bureau, Palmolive Building, Chicago. X

Science Editor: General science background in physical and biological sciences with writing and/or publishing experience. Challenging opportunity for younger man in dissemination of scientific knowledge at adult level and publishing. Salary varies with academic training and experience. Address Franklin J. Meine, Editor-in-Chief, American Peoples Encyclopedia, 153 N. Michigan Avenue, Chicago. 6/9

■ **For Classified "CHARGES and REQUIREMENTS" see page 20, June 2nd issue.**

UNIVERSITY OF DACCA, East Bengal, Pakistan.

● Applications are invited for the following posts on terms stated against each:

1. **Professor of Physics**—Salary will depend upon qualifications and may amount to Rs. 2000/- p.m. including allowances. In exceptional cases it may be increased to Rs. 2500/-p.m.
2. **Professor of Botany**—Salary will depend upon qualifications and may amount to Rs. 1500/- p.m. including allowances.
3. **Reader in Geology**—Salary and allowances up to Rs. 1000/-p.m.

(N.B. Re. 1/- = 1/6d)

Appointments will be on deputation or contract not exceeding 5 years or permanent basis in exceptional cases. Benefit of Provident Fund for service not less than five years, contribution to which on either side being 6¼% of the salary. Quarters will be provided on payment of 10% of salary. First class free passage for joining appointment at Dacca will be provided.

Applicants should be distinguished scholars, with experience of teaching and guiding research. Knowledge of English is essential. Candidates should give full details of age, nationality, academic career, teaching experience, and original publications enclosing a copy of each; also testimonials and names and addresses of three referees.

Applications must reach the Registrar, University of Dacca (P.O. Ramna) East Bengal, Pakistan not later than 31st July, 1950.

The Market Place

For "CHARGES and REQUIREMENTS" see page 21, June 2nd issue.

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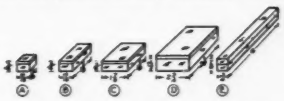

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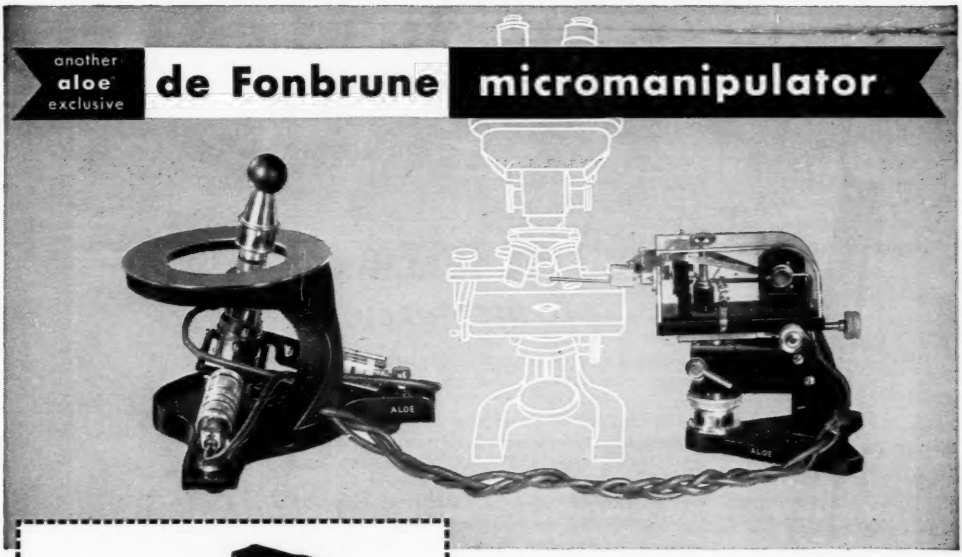

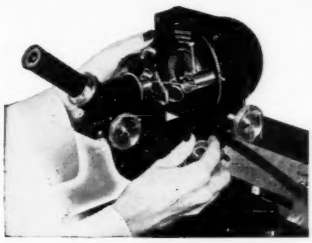

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